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PLASTIC STUDIES IN ABNORMAL RENAL ARCHITECTURE

IV. VASCULAR AND PARENCHYMAL CHANGES IN ARTERIOSCLEROTIC BRIGHT'S DISEASE

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In the studies previously published in this series Oliver and his co-workers [1] presented in plastic form the abnormalities of renal structures that develop in terminal hemorrhagic Bright's disease. The methods used were those that disclose the three dimensional aspect of the structural alterations, namely, maceration and dissection of the tissues and reconstruction of the abnormal elements by the Born wax plate method. In the present investigation, this study of Bright's disease is continued, and the first-mentioned method is used to investigate the arteriosclerotic form of this disease with the view of obtaining in this condition, also, a better understanding not only of the particular abnormalities of the units which compose the kidney but of the deviation from normal of the architecture of the organ as a whole.

In this paper no distinction is made between what is known as benign and malignant nephrosclerosis, although it became evident during the study that certain extreme and peculiar conditions develop in the kidneys in those cases which terminate in renal failure. There was no difficulty in identifying this group with that described by Fahr and others as malignant nephrosclerosis, but since essential structural changes are common to all the cases it was felt to be undesirable at present to treat this group separately. A later communication will be concerned with the topographic arrangement of the units as they are found in the kidneys in specific examples of arteriosclerotic disease, and a consideration of the varied form of the vascular disorder is deferred until that time.

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1. (*a*) Oliver, J., and Lund, E. M.: Arch. Path. **15**:755, 1933. (*b*) Oliver, J., and Luey, A. S.: Arch. Path. **18**:777, 1934; (*c*) **19**:1, 1935.

MATERIAL AND METHODS

The kidneys studied were from persons whose condition was diagnosed clinically and at postmortem examination as arteriosclerotic Bright's disease Addis and Oliver [2] presented the cases of seven of these (cases 62, 65, 66, 67, 68, 69 and 70). The kidneys of two other persons with this disease were received from Mount Sinai Hospital through the courtesy of Dr. Paul Klemperer, who has reported the case of one of them.[3]

Blocks of tissue which had been preserved in solution of formaldehyde U. S. P. were digested in concentrated hydrochloric acid until soft enough to be easily dissected—a determination which is arrived at through experience. This process took from twelve to forty-eight hours, depending on the temperature. The material was washed in several changes of water and was then dissected in water under a binocular microscope. Stereoscopic photographs were taken of the dissected specimens as they lay in the water, and these were mounted for study. The accompanying figures were drawn from both the actual specimen and its photographic reproduction. There is a uniform magnification of all the figures of × 15, as in the earlier articles of this study.

Certain difficulties are met in the presentation of data derived by the methods which have been used. For example such qualities as elasticity, turgidity, tensile strength and plasticity, which can readily be appreciated on dissection, cannot be represented in the illustrations and have therefore been included in the verbal description in as objective a form as possible. Also in the following descriptions, although for convenience the changes in the blood vessels and parenchyma have been presented separately, the relationships between these two elements of the kidney's structure were studied and are considered later in the comment.

I. ALTERATIONS IN THE BLOOD VESSELS

There is a great probability that the knowledge of the exact form of the arterial system of the human kidney has been hampered by the inadvertent use of pathologic material for anatomic investigation and by a lack of appreciation of the variation that normally occurs from one kidney to another and even between vessels of similar character within the same organ. For example, in the same kidney there may exist as so-called arcuate arteries both archlike and strictly dichotomously dividing arteries, with great variability in size, number, arrangement, location, range of distribution and direction of their branches. When, therefore, one compares the isolated portions of such vessels from normal and pathologic material caution must be used in considering the qualities of thickness, length, tortuosity and mode of branching. Any accurate conception of the direction and degree of the departure from normal must be gained by a consideration of many vessels from the same organ. Tactual as well as visual appreciation of these alterations is also of great value in determining their real significance.

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2. Addis, T., and Oliver, J.: The Renal Lesion in Bright's Disease, New York, Paul B. Hoeber, Inc., 1931.

3. Klemperer, P., and Otani, S.: Arch. Path. **11**:60, 1931.

In the matter of nomenclature the older terms have been used for designation of the arterial branches. If roentgenograms or corrosion preparations of the injected vessels or my own dissections of normal kidneys are studied it is quite apparent that the designations of interlobar, arcuate and interlobular arteries do not accurately describe or correctly locate definite divisions of the renal artery. This has been pointed out by Gross,[4] Dehoff [5] and von Möllendorff,[6] who have suggested other terms, but these have as yet received no formal acceptance or general use.

1. *Retrogressive and Degenerative Changes.*—(*a*) Variations in Shape and Size: Although the collapse and flattening of the larger vessels that occur when arteries are dissected from their supporting tissues has somewhat increased their apparent diameters, a fairly good conception can be gained from figure 22 of the thickness of the various divisions of normal renal arteries.

The larger arteries (interlobar and arcuate) in arteriosclerotic material are usually thickened (figs. 3, 11 and 23). They are also more opaque than normal, are firm and round and so preserve their contours as contrasted with normal vessels, whose walls, being extremely delicate, not only are easily torn but also collapse and flatten when dissociated from the other tissue. Thickening of the intima, a lesion so prominent in the histologic section, cannot be appreciated in dissected material unless the lumen is made visible by some fatty change in the wall, in which case variations in the diameter of the lumen appear. This is seen in figures 2 and 23.

The interlobular arteries are also firm but in addition tortuous. The course of the normal interlobular vessel is not straight and even has been described by von Möllendorf [6] and others as spiral (*schraubenförmig*). This tendency is intensified by the arteriosclerotic process, a result of the development of asymmetrical lesions in the arterial walls or of localized variations in the elasticity of its tissues. Another factor is the accommodation of the vessel that must of necessity accompany changes in the thickness of the cortex. Where the cortex is narrowed there results also a shrinking of the vessel, as may be seen by comparing figures 23 and 22. Although the main stem of the former is broken off at the end and its entire length therefore is not represented, the fact that it has decreased considerably in length can still be appreciated when it is realized that in situ it traversed the entire width of the cortex

4. Gross, L.: J. M. Research **38**:379, 1918.

5. Dehoff, E.: Virchows Arch. f. path. Anat. **228**:134, 1920.

6. von Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1930, vol. 7, p. 1.

without significant kinkings. Its branches, however, are noticeably tortuous, as are also the vessels shown in figures 9, 11 and 12.

The efferent arterioles do not normally spiral, but this effect is frequently seen in the diseased ones (figs. 1, 2, 4, 9 and 23). Arterioles are also found which have shrunk to fine threads, as shown in the tips of the interlobular branches of figure 23. The same shrinkage is to be seen in figures 24, 25 and 26.

(*b*) Degenerative Changes: One of the commonest and perhaps the most striking of the degenerative lesions encountered in the vessels of an arteriosclerotic kidney is the accumulation of fatty material that appears as a bright creamy or white substance spotting or impregnating the vessel wall. Lipoidal substances may also appear as sparkling crystals scattered along and through the walls of the blood vessels (fig. 6). The infiltration in the more severe lesion may make the wall of the vessel opaque (figs. 9, 10, 11, 12 and 23).

In the larger vessels (interlobar and arcuate) fat may appear distributed in various forms, as an isolated stellate lesion (fig. 3), as opaque plaques (fig. 10) or as a line in the intima (figs. 2 and 23).

The interlobular arteries in some examples show extensive fatty change throughout irregularly scattered long segments, but seldom is the entire vessel involved. The segment affected by this change is firm in texture, irregular in contour and is partially or completely opaque. Figures 9, 10, 11 and 12 show such extensive fatty change in the interlobular arteries with a variation in the degree of opacity.

In the afferent arterioles two points of especial interest are noted in the study of the fatty lesion. The first is that the degree of parenchymal change, to be described later, has a peculiarly close association with the lesion in these vessels. Second, the fatty lesion seems to originate in this portion of the arterial bed, for in kidneys which show a mild degree of vascular disease this portion alone is involved.

The fatty changes in the afferent arterioles and the terminal divisions of the interlobular artery vary in appearance. On corkscrew-like vessels of either sort the fatty deposit is often prominent in widened evenly spaced portions so that a rhythmic pattern results, as seen in figure 23. Or again it occurs in irregular thickenings of the vessel wall, also to be observed in figure 23 and in figures 1, 2, 3, 4 and 5. In other examples discrete nodules of fat occur in a series, resembling a string of beads (figs. 2, 3, 13, 19 and 21). These are elevated above the unaffected portions and are definitely local thickenings in the wall and not deposits on its exterior surface. Sometimes they merge together and so lose the regularity of their appearance (fig. 19). The fatty change may be followed into the very termination of the arterial system.

In glomeruli in which fatty deposits are present the affected capillaries appear shining through the capsule as bright twisted threads (figs. 8, 13 and 24). When degenerative changes have severely involved the glomerulus, the vas efferens and its capillary branches may in turn present the same fatty nodules and other lesions that appear on the afferent arterioles. This is shown in figures 2, 14 and 21.

Besides fatty involvement there also occurs a degenerative change within the vessel wall which causes it to assume a ground glass appearance as contrasted with the glaring opacity of the fatty change. Such areas can be observed in figures 8 and 10. These are found in the interlobular artery along with the accumulation of fat and dilatation. Hyaline change and necrosis of the vessel wall are observed in histologic sections of the material showing these degenerative lesions, and the ground glass appearance in the isolated specimen probably indicates one of these changes.

(*c*) Dilatation: In certain kidneys dilatations of the smaller arteries are conspicuous. The interlobular artery may show great irregularity with abrupt or gradual bulges, as is observed in the right branch of figure 11 and in figure 12. The dilatation may in addition be associated with fatty change, as seen in figures 10, 11 and 12, or with the ground glass appearance, as shown in figures 8 and 10. Smaller and more definitely circumscribed aneurysmal outpouchings are observed in some vessels, as demonstrated in figures 7 and 9. Around these varices there is marked proliferation of fibrous connective tissue, which often cannot be removed by dissection, so that the adhering shreds give the isolated vessel a ragged appearance (figs. 8 and 11). Trailing from these dilatations are thin vessels which are apparently new-formed channels, for they cannot be identified with any of the normal branches of the interlobular artery. It can be seen in figures 9 and 10 that these vessels certainly are not vasa afferentia.

The afferent arteriole may be dilated into a funnel, sausage or spherical shape. It may pinch in at the hilus or pass to the glomerulus without apparent diminution. Figure 11 shows such dilated sausage-shaped vasa afferentia with associated fatty change on the left interlobular artery. The glomeruli are not present. In figures 7 and 13 the afferent arterioles leading to deformed glomeruli are dilated as they approach them. The dilated vas afferens in figure 42 might be mistaken for another glomerulus. Further examples of dilated afferent arterioles are found in figures 10 and 32.

These severe lesions of the vessels, especially those of the interlobular arteries, are always accompanied by degenerations of the nephrons in the immediate neighborhood, and are found commonly in the more severe form of arteriosclerotic Bright's disease.

2. Development of New Vascular Paths.—In spite of the numerous studies on the vascularization of the kidney it is still being debated whether all the blood which nourishes the parenchyma of the normal organ first passes through the glomerular tufts. The reader is referred to a recent review of this subject by von Möllendorf.[6] According to the generally accepted view, the interlobular and arcuate arteries rarely have branches that do not bear glomeruli and therefore break up directly into capillaries which join the intertubular network. Whatever the relations are between the arterial supply and the intertubular network in the normal kidney, the conditions affecting the distribution of blood in the pathologic organ are entirely different. Here, with many glomeruli fibrosed and the afferent arterioles and even larger vessels occluded, rearrangements for the distribution of blood are essential, and moreover forces are present for the establishment of new relations by the opening up of formerly insignificant channels wherever they may exist as well as by the development of new ones. The need for such adaptations in the circulation of the kidney in hemorrhagic Bright's disease and a type of vascular change that accomplishes this result have been described by Oliver and Luey [1c] in a previous article of this study.

Investigators have claimed persistently that they had demonstrated four pathways by which the blood could reach the intertubular network without passing through the glomerular capillaries, and the existence of such channels has in turn been vigorously denied by others. The channels described are (1) Ludwig's vessels, i. e., a small branch from the afferent arteriole not leading to a glomerulus but breaking up into intertubular capillaries; (2) small direct branches to the tubular network from the interlobular artery; (3) direct branches from the arcuate artery or from the lower parts of the interlobular artery forming arteriae rectae verae, and (4) anastomoses between the afferent and efferent arterioles either within or outside the glomerular capsule.

In the present study of the arteriosclerotic kidney numerous examples of all these arrangements have been encountered.

Ludwig's vessel is demonstrated in figures 16 and 20. When it is developed into a significant channel for blood that has been prevented from passing through a glomerulus it is not the minute structure that has been described in the normal kidney but is as large as the vas afferens. Direct branches from the interlobular artery in the outer part of the cortex are shown in figures 9, 19, 20, 21 and 23. Direct branches from the interlobular and arcuate arteries forming arteriae rectae verae are seen in figures 2, 11, 14, 15 and 18. These are straight vessels to the medulla which break up into long branches of ever increasing fineness. They resemble exactly the vasa afferentia of the glomeruli

of the lower region of the cortex and join these vessels in the vascular fascicles which lie between the collecting tubules. All these abnormal new-formed vessels may be beaded with fat, as is shown in figures 2, 16 and 21.

A clue to the origin and evolution of these vessels is obtained by observing the vasa afferentia that lead to misshapen or contracted glomeruli, which frequently show fatty change. Apparently, union is established between the vas afferens and vas efferens so that the glomerulus is attached to the side of a continuous vessel. In figure 17 the continuity of the thickened vessel passing along the side of the glomerulus may have arisen as an anastomosis between the vas afferens and the vas efferens either by the incorporation of a part of the glomerular tuft or by effecting the formation of a union outside the capsule. In figure 18 can be seen all stages of such a change from the usual normal arrangement of distinct afferent and efferent arterioles through increasing atrophy of the glomerulus to its total disappearance with the consequent persistence of a continuous vessel. Often the site of the former glomerulus is indicated by a localized thickening of the vessel, a little tuft or a knob of fat (figs. 2, 14 and 21) or by a sharp bend a short distance from the beginning of the arteriole (fig. 18).

Besides the changes in the arterioles described, the more severely affected kidneys also show a change in the capillary network. There develops an intertwining of wiry capillary vessels, stronger, coarser and apparently more numerous than are found either in undiseased kidneys or in those areas of affected kidneys where the parenchyma is still relatively normal. Whether there is an actual increase of the vascular structure is difficult to decide, but at least the channels are so changed in character that they come into great prominence and offer a definite obstacle to dissection because of their tough springy texture. When there has been considerable shrinkage of parenchymatous elements these vessels appear to form a definite framework in which tubular and glomerular remains are supported. The capillaries are very irregular, making sudden changes in diameter from very fine, almost invisible filaments, which are attached laterally to tubules, glomeruli and arteries, to stout channels which then again may become filamentous. They anastomose freely and bind together structures such as glomeruli (figs. 24 and 26) that are normally not connected. These anastomoses are apparently the basis of such abnormalities as are seen in figure 27, in which vessels from five glomeruli unite to form one long straight channel. Figure 28 shows seven glomeruli joined in a chain. Another change in the capillary structure sometimes observed is the coarsening of the vas efferens into a stout vessel with few divi-

sions and these short and stubby at some distance from the glomerulus, as shown in figure 29. This may be compared with the vas efferens of figure 18.

II. ALTERATIONS IN THE PARENCHYMATOUS STRUCTURES

Changes in the nephron are described only briefly, as the same deviations from normal are seen here as are observed occurring in terminal hemorrhagic Bright's disease. These have been fully described and the implications discussed in previous papers.[1]

1. *Atrophy.*—In arteriosclerotic Bright's disease atrophy of the parenchyma is the most frequently occurring lesion. The atrophic change may be found extending from the glomerulus to the collecting tubule or, on the other hand, may be confined to the proximal convoluted portion. In figure 30 are shown greatly atrophied proximal tubules, some with glomeruli that do not show a corresponding diminution in size. The atrophy affecting the proximal portion is of all degrees from only a slight decrease in diameter and complexity of its convolutions, as in figure 31, to the reduction of the tubule to a tenuous thread, as shown in figure 30. The shrunken convolutions are held apart by the wiry capillary framework described previously. The atrophic nephrons do not appear to be supported by connnective tissue so much as by the vascular network or to be compressed by it so much as by each other.

The distribution of the atrophied elements in the cortex varies. In general, they are found to be most numerous in the subcapsular region and close to the large blood vessels, but they may occur singly or in irregular groups anywhere in the cortex. The effect is one of condensation of the affected nephrons into firm clumps scattered through fairly soft normal tissue. The result of this condensation is that thirty (by count) atrophied members may occupy no greater area than three normal nephrons.

When studies of the structure of these scarred areas made from stained sections were compared with the observations made by dissection it was evident that the extent of atrophy can be much better estimated by viewing the dissected material, for where the tenuous convolutions are closely packed together an appreciation of the number and continuity of the tubular structures is lost in the stained section and the general impression is obtained of an extensive proliferation of the interstitial connective tissue. On dissection, however, a typical arteriosclerotic scar is found to consist chiefly of closely packed extremely atrophic nephrons with only a slight or moderate increase in their supporting stroma.

Atrophy of the simple sort described, in varying degree, is found in the kidneys in all cases of arteriosclerotic Bright's disease, but another

and more complex kind is observed only in particular cases, namely, those in which there is a definite increase in the interstitial connective tissue. In these atrophy is not general throughout the proximal convolution, for in some parts of this portion of the tubule hypertrophy or dilatation is also present (fig. 32). The tubule has a peculiarly ragged and distorted appearance as if acted on unevenly by external forces, and if there are dilated structures in the neighborhood the whole periglomerular mass may be flattened as in figures 42, 43 and 44. As has been noted, the amount of connective tissue that surrounds these irregularly atrophied structures is definitely increased. The significance of these two forms of scarring will be discussed later.

2. *Hypertrophy.*—Although there may be some increase in size throughout the entire nephron from the glomerulus to the collecting tubule, the full extent of a definite hypertrophic process is found only in the proximal convolution. Here there is not only an increase in thickness of the tubule but multiplication and wider excursion of the coils of its periglomerular mass. Figures 33, 34 and 35 demonstrate such hypertrophic and hyperplastic changes. The increase in the diameter of the proximal convoluted tubule is often less marked near the glomerulus, which may even be much smaller than normal, as in figure 35, and becomes more conspicuous in the terminal spiral portion of Schachowa. Here hypertrophy is commonly combined with extensive hyperplastic lengthening so that reduplicating kinks and folds amplify the normally fairly straight segment. In other examples the hypertrophy occurs irregularly in parts of the proximal tubule, the remaining portions of it being normal in size, dilated or even atrophied. This combination of changes of various sorts in the tubule is seen particularly in the nephrons which are included or pass through areas of scarring in which there is a definite proliferation of the interstitial connective tissue. The presence of mixed change in the nephron, therefore, is another distinguishing characteristic of the more severe form of arteriosclerotic alteration in the kidney.

Nothing approaching the degree of hypertrophy and hyperplasia noted in the proximal convolution is observed in the remainder of the nephron. The ascending limb and distal convolution of members hypertrophied in their proximal portions may be thick and strong-looking or may not show any change. The collecting tubules which lead from these larger members are slightly thicker and firmer to the touch than those found in normal tissue. Figures 36 and 37 demonstrate the differences between collecting tubules which are atrophied, normal in size and enlarged.

3. *Fatty Changes.*—Fatty changes of varying appearance are found in all parts of the nephron. Brilliant doubly refractive crystalline particles similar to those seen in blood vessel walls appear in the glomerulus

(figs. 1 and 6), the broad ascending limb, the distal convolution and the collecting tubule (fig. 37). The distal convolution is especially the site of election for the accumulation of this substance, and here it impregnates the walls so as to make them less resilient and more plastic than normal.

Quite different in appearance from the deposits of crystalline fatty material are other accumulations of lipoid that have a shining yellow or creamy tone. They may be observed in all parts of the nephron. The glomerulus may be rendered completely or partially opaque by them. In the tubule the fatty degeneration is usually most severe in the region near the glomerulus. It may extend throughout the entire proximal tubule (fig. 39) or may affect only interrupted parts of it (fig. 40). In general, the change appears to start at the neck of the tubule and proceed distally, but sometimes this is not the case, the lower portion of the proximal convolution being affected while the upper portions are free. The fatty change is frequently accompanied by dilatation, as shown in figures 32 and 41, or by atrophy, as in figures 7 and 42. The nephrons thus affected often appear in groups, are ragged looking and disintegrate easily, and may be enmeshed in connective tissue that also contains fat.

The distal convolutions may show fatty degeneration of the wall. This is the case in the nephrons pictured in figures 40 and 42*A*. Also they are often dilated and contain white or yellowish clumps of material that in certain instances completely plug the lumen (fig. 43). Figure 42*A* shows the dilated upper part of the broad ascending limb containing fat and a fatty mass dilating the distal convolution and collecting tubule. In the dilated collecting tubules the fat may occur in a continuous mass, as can be seen in figures 42 *A* and 42 *B*, or it may form separated casts which expand the tubule locally in figure 45. It is not usually found in the lowest regions of the medulla, where the rapid union of the branches of the collecting tubules occurs, and often in this region the tubules become so attenuated that they look incapable of allowing the passage of the material accumulated above (figs. 42 *C* and 44).

In addition to all these fatty changes in the interior of the nephron, knobby protuberances are sometimes found on the outside of the walls of the proximal, the distal or the collecting tubules. These nodules seem to be at the points of capillary attachments (figs. 43 and 45).

4. *Dilatation and Obstruction.*—The dilatation and obstruction of the broad ascending limb, the distal convolution and the collecting tubule by fatty plugs have been described. These structures, especially the distal convolutions, may reach great size and press on and flatten elements with which they come in contact. They may even cause their own destruction, for the portions of tubule between the twistings and

bulgings of the extremely dilated segments may be attenuated to threads or flattened into disks. In fact, at some points they appear to be completely severed. The obstructive cause of the dilatation is not always found, since the actual point of occlusion may be in the depths of the medulla at a great distance. For example, figure 43 shows a dilated collecting tubule which was not traced into the medulla for a sufficient distance to discover the occlusion.

Dilatation is also seen in the proximal convoluted portion of the nephron and may be associated with fatty change, as shown in figures 32 and 41. Sometimes the dilatations are extremely irregular and pouchlike, as seen in figure 46. Again the dilated proximal convoluted portion is widened and flattened. The walls of the dilated tubules are thin and transparent. Figure 47 shows an enormously dilated terminal segment of the proximal tubule which passes into the broad ascending limb without any evidence of a narrow portion and which contains flocculent white material.

5. *Interrupted Tubules, Cysts and Vesicles.*—The vesicular outpouchings of portions of the proximal convoluted tubules have been noted in describing dilatation. Similar structures which are not continuous with any part of the tubule are also seen forming isolated cysts. These may be transparent or partially opaque. Also spherical, fusiform or elongated bodies, quite dense in appearance and varying in length and diameter, occur in the medulla as pictured in figure 48. They are most frequently found lying between the collecting tubules in that region where the loops of the long nephrons are situated. It is impossible to tell whether these bizarre structures are portions of Henle's loop or of the collecting tubules. In severe cases, especially in the inner portion of the cortex, the small cystic remnants of interrupted tubules appear like chains of beads, falling out of the tissue as dissection proceeds, giving no hint of their relation to the persisting nephrons. Small budlike structures projecting from the collecting tubules suggest, however, an interruption of connections with such isolated remnants (fig. 36). In the neighborhood of the distal convolution small separated cystic structures are found which resemble the budlike processes that are so frequently observed attached to this portion of the tubule. They appear to have been pinched off and now lie free in the tissue close to the tubule from which they arose. Peter [7] described such pouchlike or angular projections on the distal tubule in normal kidneys but observed that they occur much more frequently in material from aged persons. Such kidneys, of course, constantly show a certain degree of arteriosclerotic change.

7. Peter, K.: Untersuchungen über Bau und Entwicklung, Jena, Gustav Fischer, 1927.

Explanation of Plate

(This and the other illustrations used in this paper were made by E. R. Cuzzort.)

Fig. 1.—Terminal branches of an interlobular artery and afferent arterioles. The latter are stubby, heavily impregnated with deposits of fat and distorted into irregular twists and spirals. Crystalline refractile particles are present in the walls of the glomerular capsules; × 15; Addis and Oliver,[2] case 62, p. 480.

Fig. 2.—Arcuate artery with the lumen outlined with fat; afferent arterioles and arteriae rectae verae showing beadlike fatty nodules. The small indefinite mass on the left branch is the remnant of a glomerulus; × 15; Addis and Oliver,[2] case 62, p. 480.

Fig. 3.—Thickened arcuate artery with a stellate fatty lesion within its wall. Fine divisions of the interlobular branch show deposits of fat in bead formation as well as irregular individual and coalesced fatty nodules; × 15; Addis and Oliver,[2] case 67, p. 502.

Fig. 4.—Afferent arterioles deformed by fatty deposits, showing spirals and irregular thickenings infiltrated with fat; × 15; Addis and Oliver,[2] case 67, p. 502.

Fig. 5.—Interlobular artery from which most of the afferent arterioles have been torn away. Its irregular contours are pointed by small fatty accumulations; × 15; Addis and Oliver,[2] case 67, p. 502.

Fig. 6.—Terminal artery and glomeruli showing doubly refractive fat crystals, which appear as shining silver particles in the walls of the vessels; × 15; Addis and Oliver,[2] case 69, p. 510.

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EXPLANATION OF PLATE

Fig. 7.—Interlobular vessel with aneurysmal dilatations. The dilatation of one of the afferent arterioles is marked. The attached tubules show atrophy and fatty degeneration; × 15; Klemperer and Otani,[3] case 2, p. 69.

Fig. 8.—Interlobular vessel with a lesion of the ground glass type associated with dilatation and connective tissue proliferation. The efferent arteriole is first dilated, then twisted and shrunken, and leads to an atrophied glomerulus, whose capillary tuft is impregnated with fat; × 15; Addis and Oliver,[2] case 66, p. 498.

Fig. 9.—Tortuosity, fatty change and aneurysmal dilatations in an interlobular vessel. Another example of the thin vessels shown in figure 10 is found here. The deformed vessel divides to form an afferent arteriole and a capillary brush; × 15; Addis and Oliver,[2] case 65, p. 493.

Fig. 10.—Arcuate and interlobular arteries showing plaques of fat in the walls of the former and severe degenerative lesions in the interlobular branches, also dilatation associated with fatty change and a lesion with a ground glass appearance. Long thin vessels are present which have no counterpart in the normal kidney; × 15; Addis and Oliver,[2] case 66, p. 498.

Fig. 11.—Thickened arcuate and interlobular vessels. The latter are tortuous and show both extensive fatty lesions and dilatation. On the left branch are dilated afferent arterioles. A small medullary branch is present, breaking up into capillaries. Shreds of connective tissue cling to the fatty lesions; × 15; Addis and Oliver,[2] case 66, p. 498.

Fig. 12.—Enormous accumulation of fat in a tortuous and irregularly dilated interlobular vessel. Fatty nodules and areas showing less densely deposited fat are present in other parts of the vessel; × 15; Addis and Oliver,[2] case 66, p. 498.

Fig. 13.—Terminal artery bearing glomeruli which show distortion, atrophy and fatty change. The walls of the vessel show beadlike fatty protuberances. The afferent arterioles are dilated, especially the one leading to the large deformed glomerulus; through the capsule of the latter the glomerular capillaries can be seen because of fat deposits in the tuft; × 15; Addis and Oliver,[2] case 65, p. 493.


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Explanation of Plate

Fig. 14.—Branches of an interlobular artery breaking up into capillaries. Remains of glomeruli are seen on the left and right branches. Fatty change has affected the arterioles and even the capillary divisions; × 15; Addis and Oliver,[2] case 65, p. 493.

Fig. 15.—Vessel dividing to form an afferent arteriole with a glomerulus and an efferent arteriole and an arteria recta vera with long fine capillary divisions; × 15; Addis and Oliver,[2] case 69, p. 510.

Fig. 16.—Ludwig's vessel with a capillary brush. An efferent arteriole emerges from the least damaged glomerulus; × 15; Addis and Oliver,[2] case 70, p. 515.

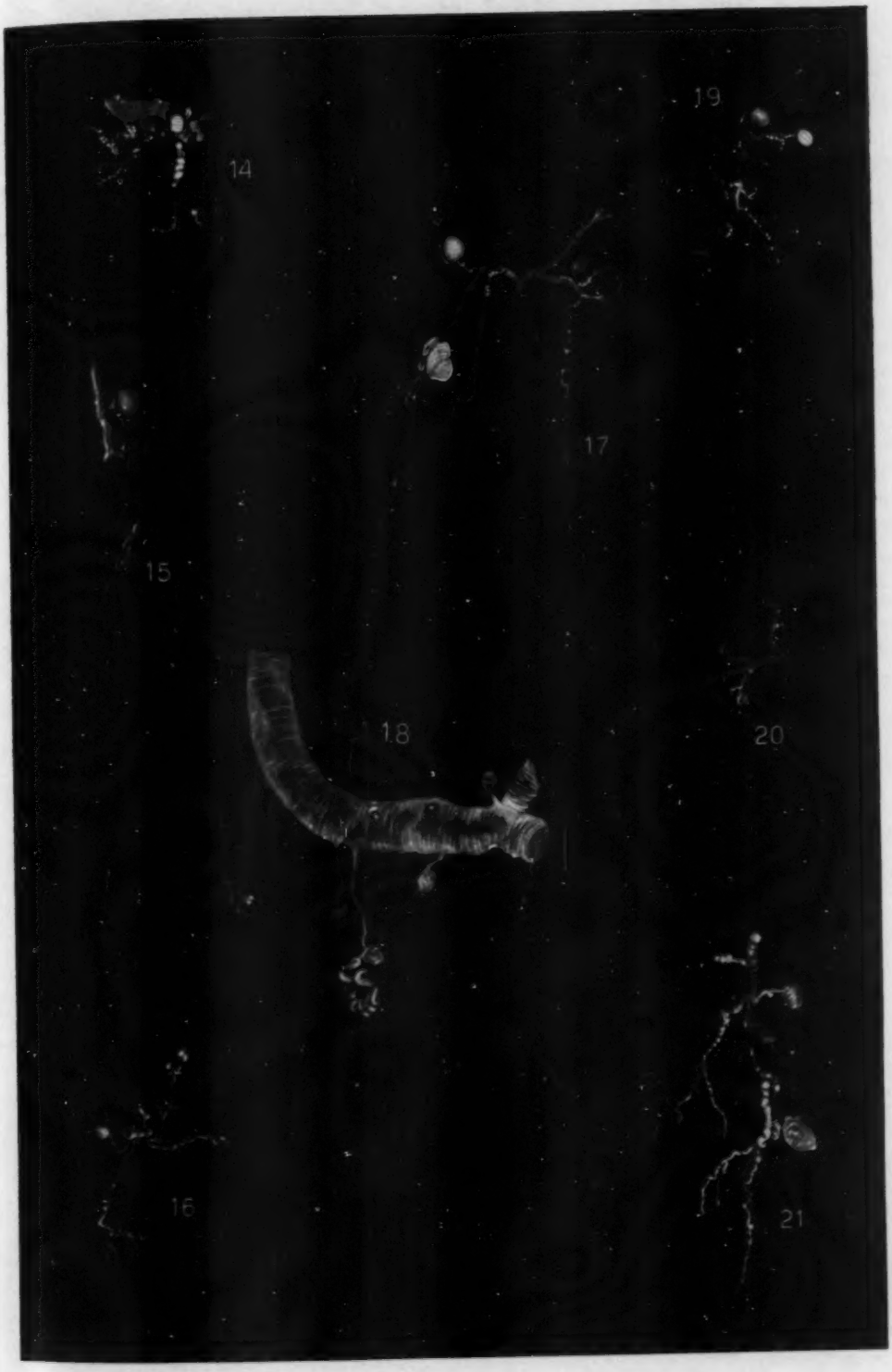
Fig. 17.—Capillary branches from the cortex arising from end branches of the interlobular artery and from efferent arterioles. One clearly shows the union of the afferent and efferent arteriole in a continuous vessel with the glomerulus attached to its side; × 15; Addis and Oliver,[2] case 65, p. 493.

Fig. 18.—Arcuate artery showing both arteriae rectae verae and arteriae rectae formed from efferent arterioles; × 15; Addis and Oliver,[2] case 68, p. 507.

Fig. 19.—Capillaries arising from vessels with no sign of formerly existing glomeruli. Other abnormalities are beading with fat and the apparent obstruction of the lumen of the main vessel; × 15; Addis and Oliver,[2] case 70, p. 515.

Fig. 20.—Interlobular vessel from the upper part of the cortex showing Ludwig's vessel and a capillary brush. The tip of the interlobular artery also divides into capillary branches; × 15; Addis and Oliver,[2] case 68, p. 507.

Fig. 21.—Interlobular artery ending in capillaries. Severe fatty changes in beadlike formation thickens the arterioles and extends to the capillaries. One of these vessels shows a sharp bend and thickening and the other a tuft, which are all that remain of the glomeruli. One glomerulus gives rise to an efferent arteriole in the normal fashion; × 15; Addis and Oliver,[2] case 62, p. 480.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Explanation of Plate

Fig. 22.—Interlobar, arcuate and interlobular arteries from a normal kidney. The arcuate artery is not arch-shaped, and the interlobular arteries branch from it dichotomously; × 15.

Fig. 23.—Thickened arcuate and interlobular vessels with fatty deposits in the intima of the former. The terminal branches of the interlobular artery show many kinds of change: tortuosity, changes in diameter, spiral turns, deposits of fat in rhythmic pattern or in irregular masses, diminution of terminal branches to tenuous threads and obliteration of the lumen with opaque fat. Vessels are present which end directly in capillary branches without intervening glomerular tufts. Most of the glomeruli are contracted, some to minute structures, and show extreme fatty change; × 15; Addis and Oliver,[2] case 70, p. 515.

Fig. 24.—An example of new capillary structure. The capillaries joining three glomeruli arise from an efferent arteriole. Two other glomeruli in this figure are also united by a capillary vessel; × 15; Addis and Oliver,[2] case 68, p. 507.

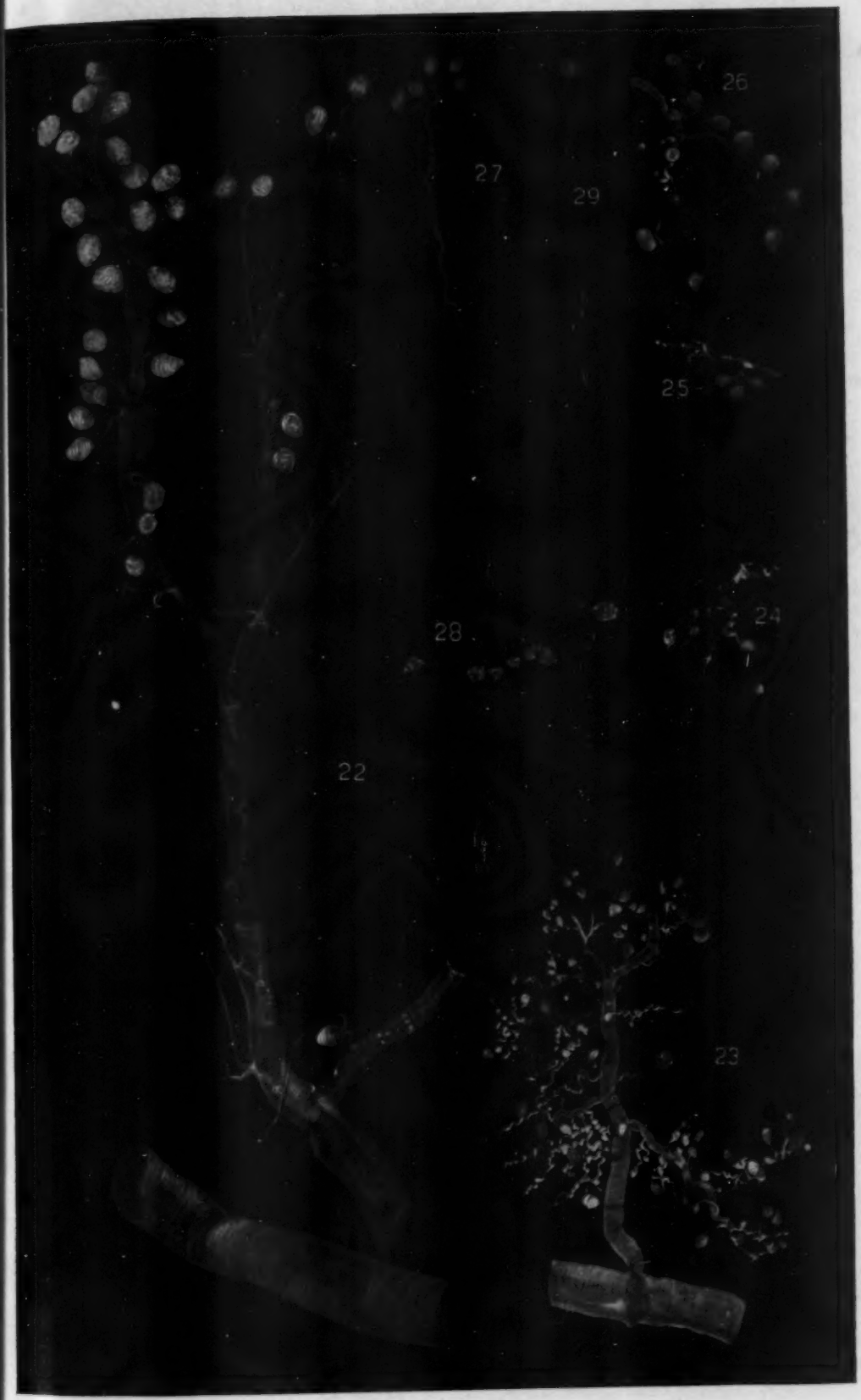
Fig. 25.—Three atrophied glomeruli on a shrunken branch of an interlobular artery; × 15; Addis and Oliver,[2] case 68, p. 507.

Fig. 26.—Glomeruli joined to each other by a new capillary structure as in figure 24; × 15; Addis and Oliver,[2] case 68, p. 507.

Fig. 27.—New vascular channels from five glomeruli uniting into a coarse vessel, which divides again into further branches; × 15; Addis and Oliver,[2] case 68, p. 507.

Fig. 28.—New capillary structure joining seven glomeruli in a chain; × 15; Addis and Oliver,[2] case 66, p. 498.

Fig. 29.—Coarse efferent arteriole with few divisions; Addis and Oliver,[2] case 67, p. 502.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Explanation of Plate

Fig. 30.—Simple atrophy of glomeruli and proximal convoluted tubules. Some of the glomeruli are greatly reduced in size; others do not show atrophy corresponding to that of the tubules; × 15; Addis and Oliver,[2] case 68, p. 507.

Fig. 31.—Simple atrophy of a glomerulus and proximal convoluted tubule, a nephron in miniature without other degenerative change; × 15; Addis and Oliver,[2] case 67, p. 502.

Fig. 32.—Irregular atrophy of proximal convolutions combined with dilatation and fatty degeneration. The afferent arterioles are dilated; × 15; Addis and Oliver,[2] case 65, p. 493.

Fig. 33.—Hypertrophied proximal tubule with a portion of the ascending limb, the distal convolution and the collecting tubule. The afferent arteriole and part of an interlobular artery are connected with the glomerulus; × 15; Addis and Oliver,[2] case 70, p. 515.

Fig. 34.—Hypertrophy of the terminal segment of a proximal convolution. Note the hyperplastic lapping and kinking; × 15; Addis and Oliver,[2] case 70, p. 515.

Fig. 35.—Hypertrophied proximal convoluted tubules with small glomeruli. Nodules of fat are seen on the outside; × 15; Addis and Oliver,[2] case 65, p. 493.

Fig. 36.—An atrophic collecting tubule contrasted with one somewhat larger than any found in normal kidneys. The latter is continuous with two strong distal convolutions and connecting pieces and one which has been interrupted at the place where the budlike formation is seen; × 15; Addis and Oliver,[2] case 70, p. 515.

Fig. 37.—Distal convolutions and collecting tubules of normal size, with walls impregnated with glittering material. The small bodies are pinched-off projections of the distal convoluted tubules; × 15; Addis and Oliver,[2] case 62, p. 480.

Fig. 38.—Nephron showing sparkling crystals or doubly refractive lipoid in the terminal segment of the proximal convolution, similar to that seen in figure 37; × 15; Addis and Oliver,[2] case 69, p. 510.


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EXPLANATION OF PLATE

Fig. 39.—Nephron showing mild fatty degeneration throughout the entire proximal convoluted tubule; × 15; Addis and Oliver,[2] case 68, p. 507.

Fig. 40.—Complete nephron and a portion of the collecting tubule, showing unconnected areas of fatty change. Fatty degeneration is seen in the distal convolution and another nephron joining the same collecting tubule; × 15; Addis and Oliver,[2] case 66, p. 498.

Fig. 41.—Nephron with a greatly enlarged glomerulus showing fatty change. The proximal convoluted tubule shows dilatation and fatty degeneration. The hypertrophic change which has occurred probably preceded these; × 15; Mount Sinai case 8562.

Fig. 42.—*A*, *B* and *C*, interlobular artery, complete nephron and complete collecting tubule. The afferent arteriole is tremendously dilated and shows shreds of adherent connective tissue. In *A* the glomerulus is enlarged and shows fatty change. The proximal convolution is atrophied. The ascending limb is dilated and shows fatty degeneration. The distal convolution is greatly dilated and plugged with fat which (see *B*) extends for a long distance into the collecting tubule. The lower medullary portion of the collecting tubule (*C*) is very attenuated; × 15; Klemperer and Otanj,[3] case 2, p. 69.


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42C
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Explanation of Plate

Fig. 43.—Those parts of a nephron lying in the cortex, with a portion of an interlobular artery and efferent arteriole. The glomerulus and proximal convolution are atrophied; the ascending limb contains fat, which is closely packed and dilates the distal convoluted tubule. The collecting tubule is dilated but empty. Fatty nodules are seen on the outside of the collecting tubule; × 15; Addis and Oliver,[2] case 65, p. 493.

Fig. 44.—Dilated distal convolutions and collecting tubule packed with fatty masses which have caused distortions and atrophy of neighboring structures. The atrophied glomeruli and proximal tubules associated with these dilated portions are lying near them. The collecting tubule becomes greatly attenuated in its medullary portion; × 15; Klemperer and Otani,[3] case 2, p. 69.

Fig. 45.—Dilated collecting tubule containing casts. The remaining collecting tubules do not show dilatation but have fatty nodules spotting their walls; × 15; Mount Sinai case 8562.

Fig. 46—Cystic dilatations of the terminal segment of a proximal convoluted tubule; × 15; Addis and Oliver,[2] case 65, p. 493.

Fig. 47.—Greatly dilated folds of the terminal portion of the proximal convolution containing flocculent white material. The part preceding the dilatation is atrophic; × 15; Addis and Oliver,[2] case 70, p. 515.

Fig. 48.—Cysts and segments of interrupted tubules; × 15; Addis and Oliver,[2] case 69, p. 510.


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III. ALTERATIONS IN INTERSTITIAL TISSUE

A connective tissue investiture occurs around the larger vessels in normal kidneys, but in arteriosclerotic material this is increased and so closely connected with the adventitia that it cannot be dissected from it without damaging the vessels. In this connective tissue envelopment many atrophied glomeruli and tubules are found. Also vestiges of glomeruli for which a connecting tubular structure cannot be located are observed on branches of the arcuate arteries. The tips of the interlobular arteries are often so inextricably embedded in connective tissue that the afferent arterioles are difficult to distinguish and so are often torn off the parent vessel in the course of the dissection. This accounts for the stripped appearance of the interlobular artery in figure 5.

Proliferation of connective tissue around the more striking vascular lesions such as dilatation, degeneration of the interlobular arteries and afferent arterioles has been mentioned. Where this connective tissue proliferation occurs around such lesions the glomeruli and tubules are frequently enmeshed and compressed by it. Also there is an accompanying connective tissue growth about the tubules that show fatty degeneration.

The stroma in regions where nephrons show simple atrophy is composed chiefly of interweaving capillaries, the fibrous connective tissue proliferation in these cases being confined to an envelopment and condensation around afferent arterioles and interlobular arteries. As will appear in a later communication in which the architectural types of contracted kidneys will be described, the significance of these two types of arteriosclerotic scarring is of considerable importance in the interpretation of the pathogenesis of the forms of arteriosclerotic Bright's disease.

Wherever connective tissue occurs there may be scattered fine particles of fatty substance. These may be silvery and glittering or dull and yellowish. Collections of larger amounts of fat are observed especially in the connective tissue that immediately surrounds blood vessels.

COMMENT

As has been shown, arteriosclerotic kidneys present combinations of destructive and constructive changes, and the latter are often adaptive in nature.

In considering the destructive changes one notes marked variations in effect depending on the location of the vascular lesion. Where the end branches of the interlobular arteries, the vasa afferentia and the glomerular capillaries are the vessels chiefly concerned, the disturbance is a local one, and uniform simple atrophy of the nephrons occurs singly or in small clumps but not in wedge-shaped sectors. As the process seizes more and more of the arterioles, more of the parenchyma

becomes useless. Many of the atrophied members, although probably decreased in functional ability, remain viable owing to the setting up of a collateral circulation.

If, however, the blood is cut off by obliterative change in the main stem of the interlobular artery the involved nephrons lie in wedge-shaped groups. Presumably if there is a slow occlusion a simple atrophy occurs, whereas if blood is suddenly cut off severe degenerative lesions, such as fatty change, may result. A large number of nephrons may be thrown out of function at one time, and there is a less immediate source of collateral circulation than obtains when the afferent arterioles or glomeruli are attacked singly. Also if the occlusion is rapid there is little time for the development of new vascular channels or for compensatory hypertrophy. Other destructive processes may now complicate the situation: The growth of fibrous connective tissue stimulated by parenchymal and vascular destruction [8] and the accumulation of débris that obstructs and dilates tubules bring about distortions of the nephrons. In this manner there develops the irregular and uneven atrophy that has been described as typical of the more severe form of arteriosclerotic Bright's disease.

Both arterioles and arteries may be affected by either slow or rapid occlusion, so that in the same kidney there may be either simple or irregular atrophy of nephrons and acute degeneration.

The adaptations which lead to the maintenance of function as portions of the parenchyma deteriorate are hypertrophy of the nephron, the reorganization of old vessels and the development of new ones.

The blood cut off by the occlusion of the afferent arteriole or the intraglomerular capillaries is received by neighboring nephrons through their own undamaged vessels and possibly also through new channels,[8] increasing their nutriment and activity, stimulating them to hypertrophy. The hypertrophy of these nephrons compensates for the loss of those damaged by vascular obstruction if this change does not occur too rapidly. However, it seems unlikely that hypertrophy would occur until enough of the tissue is affected so that a burden is thrown on the remaining tubules. This would explain the finding of the greatest hypertrophy associated with the greatest amount of atrophy.

The development of a new intertubular capillary network, probably using old pathways as well, also distributes the blood to the atrophied members and nourishes them enough to prevent their death—a problem which caused considerable discussion at the Strasbourg conference.[9]

It is with some hesitation that one refers to Ludwig's vessel, the direct branches of the interlobular artery which break up to enter the

8. It is also possible that activity in itself attracts to the nephrons that are functioning so vigorously some of the new vessels which are developing as a result of the blockage of accustomed paths.

9. Verhandl. d. deutsch. path. Gesellsch. (supp.) **15**:226, 1912.

capillary network or the arteriae rectae verae as "new vessels," since they have been described so often. However, their existence in the normal kidney in any but negligible numbers is at present denied by the majority of investigators, whereas, as I have shown, in the kidney that is significantly diseased they are a constant and frequent finding and cannot but be regarded as an active response to vascular destruction. It is believed that the past disagreement concerning the existence of these vessels in the normal kidney has arisen partly because the technic of injection into vessels under pressure was used for most of these studies. Differences in findings may be attributed to differences in pressure or to the obscuring of detail that may result from the diffusion of the injected mass through the tissues. The method of mounting and studying material prepared by such a technic has not the advantage of separating closely mingled and therefore confusing structures that is afforded by the isolation method. Another defect has been the use of pathologic material for the earlier anatomic work. For example, Virchow [10] supported his belief in the existence of arteriae rectae verae as a part of the normal renal vascularization by citing the finding that in an amyloid kidney in which an opaque substance had been injected, the medullary vessels showed complete filling while the glomerular tufts remained empty. The fact that Virchow found that arteriae rectae verae exist in amyloid kidneys shows that these are present in the pathologic organ but does not prove that they are present in the normal one. If one considers the fact that arteriosclerotic lesions develop in all kidneys and that the change may begin in early life it becomes evident that the formation of these new vessels may well be a constant phenomenon of increasing age.

There is also disagreement as to whether there are anastomoses between the vas afferens and the vas efferens either outside of or within the glomerular capsule in the normal kidney. The reader is referred to the work of Golubew [11] and Kosugi,[12] which supports the former possibility, and that of Borst,[13] who pictured anastomoses within the capsule. This subject received detailed consideration by von Möllendorf, who doubted the existence of any such connections. However, under the altered conditions which obtain when fibrosis of the capillary loops within the tuft occurs it is possible that the blood is redirected either through one short loop which connects directly the afferent and the efferent arteriole as described by Thoma [14] or through a preexisting anastomosis between the capillaries of the tuft (Borst), which becomes

10. Virchow, R.: Virchows Arch. f. path. Anat. **12**:310, 1857.
11. Golubew, W. Z.: Internat. Monatschr. f. Anat. u. Physiol. **10**:541, 1893.
12. Kosugi, T.: Beitr. z. path. Anat. u. z. allg. Path. **77**:31, 1927.
13. Borst, J. G. G.: Ztschr. f. mikr.-anat. Forsch. **23**:455, 1931.
14. Thoma, R.: Virchows Arch. f. path. Anat. **71**:227, 1877.

enlarged with use. The blood might also pass through a preexisting anastomosis between the afferent and the efferent arteriole outside the capsule (Kosugi) or through a union arising de novo, such as I have described, in response to altered conditions. The final result of any of these changes would be the single straight vessel with its glomerular appendage as described in the foregoing text and pictured in figure 17, with the functional result that the blood reaches the intertubular network in spite of the obliteration of the glomerular tufts. The frequent presence of contracted or practically nonexistent glomeruli attached to these vessels is evidence that they have arisen from a definitely pathologic mechanism. Similar glomerular remnants were described by Huber [15] and Lee-Brown [16] as present near the medulla in what was supposedly normal material and were interpreted as developmental anomalies, but the constancy of their occurrence in the arteriosclerotic contracted kidney suggests that they are effects of vascular disease. As I have mentioned, any kidney might show such changes.

The coarse and profuse capillary network that joins together all the various structural elements of the kidney accommodates the blood which has been diverted through the new channels by the fibrosis of the finer arterioles and glomerular capillary bed so that contact of the parenchymatous elements with the blood is reestablished. These adventitious capillaries are attached to the glomerular capsule at a number of points as well as to all parts of the tubules. The existence of this new irregular vascular arrangement was reported in detail by Thoma [14] in the condition designated by him as chronic interstitial nephritis. He also ascribed to pathologic change the frequent occurrence of Ludwig's vessel in this condition and of the branches of the interlobular artery which do not show glomeruli.

All these adaptive changes in the blood vessels are of the greatest importance since where they are effective the kidney can continue to function even when there has been fibrosis of the majority of the glomeruli. The condition of a kidney at any stage of the arteriosclerotic disease is, then, the resultant of quantitative changes affecting blood supply. These changes are determined by the size of vessel occluded, the extent of the lesion, the number of vessels involved and the rate of change. These quantities occurring in different combinations cause the appearance of one arteriosclerotic kidney to vary from another. The tempo of the disease determines whether the progressive processes (hypertrophy of the nephron; development of new vessels) can successfully counter the regressive processes. The continued function of the organ depends on this balance.

15. Huber, G. C.: Am. J. Anat. **6**:391, 1907.
16. Lee-Brown, R. K.: Arch. Surg. **8**:831, 1924.

VIABILITY OF CELLS IN INFLAMMATORY EXUDATES

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Most of the observations on the cellular response to inflammation have been concerned with the origin and time of appearance of the cells. Less attention has been directed to the viability and ultimate fate of the various types of cells once they have appeared on the scene. Since any precise determination of the state of viability of cells in exudates is impossible in stained smears or in sections, the cells must be observed while still fresh. Schilling [1] reported that the death of leukocytes could be determined by cessation of movement of cytoplasmic granules on dark-field examination. Lewis and McCoy [2] in an extensive study have shown that the death of cells can be accurately determined in preparations supravitally stained with neutral red. Stewart and Long [3] have used Lewis' criteria in studying the death of cells in pleural exudates of the rabbit in relation to anaphylaxis, and Scott and Finland [4] made an extensive study of the cytology of pleural effusions in pneumonia with a supravital technic.

MATERIAL AND METHODS

The present report is concerned with studies of the viability of exudate cells throughout the duration of several types of inflammatory processes. For the most part rather fluid exudates were studied, so that by repeated sampling a continuous record of changes in the cytologic pattern could be obtained at all stages of the process.

The following technic was used in making supravital differential counts, two stock solutions being kept ready for use:

A. Locke's solution

	Percentage
Sodium chloride	0.900
Calcium chloride	0.024
Potassium chloride	0.042

From the Department of Surgery and the Department of Medicine, the University of Chicago.

1. Schilling, V.: Folia haemat. **6**:429, 1908.
2. Lewis, W. H., and McCoy, C. C.: Bull. Johns Hopkins Hosp. **33**:284, 1922.
3. Long, P. H., and Stewart, F. W.: Am. J. Path. **2**:91, 1926.
4. Scott, T. F. M., and Finland, M.: Am. J. M. Sc. **188**:322, 1934.

This is adjusted to p_H 6.6 with a tenth-normal solution of sodium carbonate and buffered with a 2 per cent solution of a standard fifteenth-molar phosphate buffer at p_H 6.6.

B. A 1.26 per cent solution of ammonium chloride.

A solution consisting of nine parts of buffered Locke's solution to one part of ammonium chloride solution is warmed in the incubator. Four cubic centimeters is placed in each of two Wassermann tubes, and 0.04 cc. of a 1 per cent aqueous solution of neutral red is added to each, giving a concentration of 1 : 10,000 of the dye. Two drops of exudate obtained by aspiration are dropped into this solution, and the tubes are gently inverted several times to make an even suspension. Counts are made immediately with the use of a blood-counting chamber in a humidified warm box. As a routine 100 cells are counted from each sample, and the numbers of dead and living cells of each type are tabulated. Stained smears of whole exudate are also made to check the supravital differential count.

Dead cells can readily be distinguished by the appearance of the nucleus, which is granular and is usually stained diffusely red. In the presence of ammonium salts the cytoplasmic granules and vacuoles in the living cell remain yellowish, so

TABLE 1.—*Average of Viable Cell Count in Exudate Produced by Aleuronat in Five Rabbits*

Time in Days	Percentage of Polymorphonuclears		Percentage of Other Types of Cells	
	Living	Dead	Living	Dead
¼	90	3	6	1
1	81	2	15	2
2	51	4	38	7
3	47	6	40	7
4	37	6	42	15
5	27	8	45	20
7	21	5	52	22

that a large vacuole or aggregation of granules cannot be mistaken for a dead nucleus. While the use of a blood-counting chamber precludes differential counting with the oil immersion objective, we feel that the convenience of ruled areas for counting and the avoidance of possible trauma to cells from the cover slip outweigh the disadvantages of the method. A constant p_H, slightly on the acid side of neutrality, as recommended by Olesen and Thomsen,[5] insures that the pinkish-red color of dead and dying nuclei will be distinct.

OBSERVATIONS

Exudates Produced by Aleuronat.—The cell picture was followed in exudates produced by the injection of from 5 to 10 cc. of an aleuronat-broth mixture into the pleural cavity of rabbits. Daily specimens were obtained by intercostal puncture.

In a series of five experiments (table 1) few dead cells were present during the first twenty-four hours. From the third to the seventh day the percentage increased progressively from 13 to 27. Mononuclears, macrophages and lymphocytes appeared in large numbers in two days, and their number increased to 74 per cent of the total count by the seventh day.

As the polymorphonuclear leukocytes died they became ingested by large phagocytes. From the third day on, numerous dead, intact or fragmented poly-

5. Olesen, M., and Thomsen, O.: Ugesk. f. læger **95**:886, 1933.

morphonuclears were seen within macrophages. Since these could not be accurately counted and tabulated, the actual death of polymorphonuclears is much greater than the percentage given, particularly in the later stages of the inflammatory process.

In one experiment aleuronat broth was injected into both pleural cavities of a rabbit. The average viable cell count for the two sides is shown in table 2. Particular care was taken in an attempt to distinguish between monocytes and macrophages. From the second to the seventh day the monocyte count remained between 29 and 44 per cent, while the macrophage count increased from 12 to 33 per cent.

Streptococcic Empyema.—Two rabbits were given intrapleurally 0.2 cc. of a fresh broth culture of Streptococcus viridans, freshly isolated from a patient with

TABLE 2.—*Average of Viable Cell Count in Exudate Produced by Aleuronat in the Left and the Right Pleural Cavity of a Rabbit*

	Percentage of Polymorphonuclears		Percentage of Monocytes		Percentage of Macrophages		Percentage of Lymphocytes	
Time in Days	Living	Dead	Living	Dead	Living	Dead	Living	Dead
¼	93	2	1	0	0	0	3	1
1	83	1	7	2	1	3	3	0
2	45	5	29	8	10	2	1	0
3	37	6	30	6	17	2	2	0
4	36	4	26	3	23	4	3	1
5	19	3	36	5	19	7	8	3
6	14	3	35	9	22	11	4	2
7	15	5	25	12	26	6	6	5

TABLE 3.—*Average of Viable Cell Count for Five Rabbits with Pneumococcic Empyema*

	Percentage of Polymorphonuclears		Percentage of Monocytes		Percentage of Macrophages		Percentage of Lymphocytes	
Time in Days	Living	Dead	Living	Dead	Living	Dead	Living	Dead
¼	94	2	1	0	0	0	3	0
1	83	1	4	1	1	0	9	1
2	73	1	8	1	9	0	7	1
3	61	2	16	0	10	0	10	1
4	57	5	13	2	8	1	13	1
5	57	4	19	3	4	2	10	1
7	56	2	9	0	9	1	20	3

empyema. In the first animal the number of living polymorphonuclear leukocytes fell from 86 per cent at six hours to 74 per cent at three days as the number of dead polymorphonuclears rose from 7 to 9 per cent. The exudate on the third day showed only 14 per cent living monocytes, 1 per cent dead monocytes and 1 per cent living macrophages. The rabbit died the evening of the third day. The second rabbit showed a decline in the polymorphonuclear count from 95 per cent at 1 day to 87 per cent at 2 days. Only 2 per cent of dead polymorphonuclears were seen. The rabbit died the night of the second day. The autopsies revealed massive empyema. In spite of an overwhelming infection the number of dead cells in the exudate was not increased over that seen in the exudates produced with aleuronat. However, the ingress of large phagocytic cells was apparently retarded.

Pneumococcic Empyema.—Acute empyema was produced in five rabbits by the intrapleural injection of 0.02 cc. of a twenty-four hour broth culture of Pneu-

mococcus type II of low virulence[6] in 10 cc. of saline solution. The percentage of dead cells, which reached a maximum of 10 (table 3), was not nearly as great as in the sterile exudate produced by aleuronat. On the other hand, the relative number of living polymorphonuclears remained higher, declining gradually from 94 per cent at six hours to 57 per cent on the fourth day. The ingress of large phagocytes followed the same general qualitative pattern as that seen in the exudates produced by aleuronat, but in much smaller numbers. The percentage of lymphocytes was somewhat greater in the later stages.

Experimental Pneumonia.—Through the agency of Dr. Robertson and his collaborators,[7] the viability of exudate cells was studied in a series of twenty-seven dogs with experimental pneumococcic pneumonia of various stages. The dogs were killed at various stages of the disease by the intravenous injection of pentobarbital sodium or by electrocution. Immediately after death a specimen of consolidated lung from 2 to 3 cc. in size was obtained and washed free from blood in Locke's solution. Small fragments from the center were excised with fine scissors and gently shaken in 4 cc. of the solution used in supravital staining. Counts were made from the supernatant suspension. The results in nine of the dogs with representative lesions are summarized in table 4. The reliability of this method is shown by comparing the relative numbers of macrophages and polymorphonuclears with the cellular picture seen in microscopic sections.

In the series of twenty-seven dogs it was found that during the first day of the disease less than 10 per cent of the exudate cells were dead. The number of dead cells increased after from twenty-four to forty-eight hours until by the third or fourth day the percentage had risen to from 20 to 25 per cent. One dog with a lesion in the stage of beginning resolution showed 36 per cent of dead cells.

Coincident with the aging of the lesion and the increase in the number of dead cells the cyto-architecture of the exudate changed. During the first day almost all the cells were polymorphonuclears. After twenty-four hours mononuclears and macrophages appeared in increasingly greater numbers. Pulmonary lesions in the stage of resolution all showed a relatively high percentage of cells of the mononuclear-macrophage type. Lymphocytes did not appear in significant numbers. These changes in the suspensions of vitally stained cells correspond with the changing cytologic characteristics of the microscopic picture as the lesion approaches the stage of recovery. This cellular transformation of the parenchyma of the lung and intra-alveolar exudate accompanying recovery and resolution of the lesion has been described by Robertson and his co-workers.[8] Briefly, it consists of an increase in the number and size of large mononuclear cells in the alveolar walls, which become detached from the septums and enter the air spaces, where they assume the appearance and functions of the free histiocyte or macrophage.

Abscesses.—Abscesses were produced in five rabbits by the intramuscular injection of Staphylococcus aureus suspended in a 0.5 per cent solution of calcium chloride. In one animal, which was followed from the second to the seventeenth day,

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6. This strain of Pneumococcus type II was obtained from the Hospital of the Rockefeller Institute and is known as D 39 (Woo, S. T.: J. Exper. Med. **43**:623, 1926).

7. Robertson, O. H.; Coggeshall, L. T., and Terrell, E. E.: J. Clin. Investigation **12**:467, 1933.

8. Robertson, O. H.; Coggeshall, L. T., and Terrell, E. E.: J. Clin. Investigation **12**:433, 1933. Coggeshall, L. T., and Robertson, O. H.: J. Exper. Med. **61**:213, 1935.

the number of polymorphonuclears fell from 85 to 8 per cent as the number of large phagocytes rose from 15 to 78 per cent. The count of viable cells showed the following changes from the second to the seventeenth day: The percentage of living polymorphonuclears decreased from 20 to 1, and that of dead polymor-

TABLE 4.—*Viable Cell Counts for Nine Dogs with Experimental Pneumococcic Lobar Pneumonia* *

Dog	Age of Lesion	Percentage of Polymorphonuclears		Percentage of Monocytes		Percentage of Macrophages		Percentage of Lymphocytes		Character of Lesion	
		Living	Dead	Living	Dead	Living	Dead	Living	Dead	Gross Appearance	Microscopic Appearance
1	6 hours	Percentage of all cells: living, 99; dead, 1								Early process	Polymorphonuclear exudate; no macrophages
2	18 hours	87	9	1	0	0	0	2	1	.....	Polymorphonuclear exudate; no macrophages
3	24 to 36 hours†	78	14	1	1	1	1	3	1	Red hepatization	Beginning focal macrophage reaction
4	48 hours	74	18	3	2	0	0	3	0	Red hepatization	Beginning focal macrophage reaction
5	Third day	Percentage of all cells: living, 77; dead, 23								Red hepatization	Polymorphonuclear exudate; a few macrophages
6	Fourth day‡	45	15	6	6	18	2	5	3	Beginning resolution	Marked macrophage reaction
6	48 hours	71	12	6	5	1	0	4	1	Red hepatization	Polymorphonuclear exudate; no macrophage reaction
6	24 hours	77	14	1	5	1	0	1	1	Early process	Polymorphonuclear exudate; no macrophage reaction
7	Fifth day§	74	19	0	1	0	1	2	3	Red hepatization	Polymorphonuclear exudate; no macrophage reaction
8	Sixth day; animal recovering	38	10	5	7	25	6	8	1	Beginning resolution	Marked macrophage reaction
9	Seventh to eighth day; 3 to 4 days after recovery	30	4	12	1	38	3	9	3	Resolution	Marked macrophage reaction

* In the majority of the dogs the lesion was in the lower lobe of the right lung. In some it was in the middle lobe of the right lung or in the lower lobe of the left lung.

† At this stage macrophages are still attached to alveolar walls, and the cellular exudate is predominantly polymorphonuclear in character.

‡ This dog was killed on the fourth day of disease and showed lesions from twenty-four hours to four days old.

§ This animal died.

phonuclears, from 65 to 7, while the percentage of living mononuclear cells increased from 9 to 18, that of dead mononuclear cells from 6 to 70, that of living lymphocytes from 0 to 2 and that of dead lymphocytes from 0 to 2. Mononuclears and macrophages were included in one group.

In another rabbit an abscess of seven weeks' duration was incised and drained. Over the course of five days the percentage of living polymorphonuclears increased from 7 to 75 and the percentage of dead polymorphonuclears, from 5 to 12, so that the draining material assumed the characteristics of a fresh exudate.

Three abscesses of one, four and five weeks' duration, respectively, showed on aspiration from 0 to 5 per cent living polymorphonuclears and from 5 to 10 per cent dead polymorphonuclears. About two thirds of the remaining cells, which were chiefly large phagocytes, were dead.

Empyema in Human Beings.—Viable cell counts were made repeatedly in three cases of empyema in human beings. In one case the number of dead polymorphonuclears in exudate obtained by aspiration increased from 14 per cent on the third day of the disease to 25 per cent on the fifth day, as the total percentage of polymorphonuclears remained about 85. Six weeks after adequate drainage the percentage of dead polymorphonuclears was 11. In the second case, one of empyema of several weeks' duration, the count showed 42 per cent dead cells of a total of 59 per cent polymorphonuclears. Four days after open drainage was instituted only 15 per cent of a total of 85 per cent polymorphonuclears were dead. In the third case, in which the condition occurred three weeks after the onset of lobar pneumonia, 45 per cent of a total of 76 per cent polymorphonuclears were dead. After four daily tappings, with relief of symptoms, only 9 per cent of a total of 89 per cent polymorphonuclears were dead. Thus in human empyema the exudate, after drainage of one sort or another, shows the picture of a fresh process, at least as far as the viability of the polymorphonuclear leukocyte is concerned.

The counts in these cases, as well as a number of isolated counts, have demonstrated that in early or progressing infections the percentage of living polymorphonuclears is high—from 80 to 90. In older and more localized exudates from one fourth to two thirds of the polymorphonuclears are dead, and the proportion of large phagocytes is increased. Schär [9] has reported somewhat similar observations in peritoneal exudates.

COMMENT

The repeated determination of the number of dead cells at various stages of the inflammatory process supplies some information concerning the life span of these cells. The findings reported in this paper suggest that polymorphonuclear leukocytes remain alive on an average of from two to six days after arriving in the pleural cavity or the pulmonary alveoli. In the case of intramuscular abscesses, in which the conditions for nourishment and exchange of gases presumably are less advantageous, death occurs much sooner.

Our experiments cast little light on whatever causes may be active in producing the death of cells. It is interesting to observe, however, that leukocytes died just as rapidly, if not more so, in a sterile exudate produced by aleuronat as in an exudate produced by pathogenic microorganisms.

SUMMARY

The viability of the various types of exudate cells in acute inflammatory processes was studied by a supravital staining technic. After about forty-eight hours the polymorphonuclear leukocytes begin to die. Coincident with their death a gradually increasing ingress occurs of cells of the mononuclear-macrophage type.

9. Schär, W.: Deutsche Ztschr. f. Chir. **210**:250, 1928.

SOMATIC CARCINOMA AND THE STATE OF THE INTERSTITIAL CELLS OF THE TESTICLE

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The relations of malignant tumors to endocrine glands and of the latter to the former have led to many publications. The present study is concerned with the possible relation of carcinoma in any part of the body to the state of the interstitial cells of the testicle.

Sehrt [1] investigated this relation in man. He concluded from a study of 21 cases that there is a constant and significant degree of hyperplasia of the interstitial cells of the testicle in 79.2 per cent of persons having carcinoma. This hyperplasia is manifest in the presence of large masses of cells not only near destroyed tubules but in areas with normally functioning tubules and near the tunica albuginea. The latter frequently contains small heterotopic groups of interstitial cells. Whereas, according to Sehrt, the testicle when "normal" contains interstitial cell deposits of from 10 to 30 cells, in several of the cases of carcinoma it showed groups numbering 1,000 or more cells. He believed that the hyperplasia was so striking and characteristic that the presence of carcinoma could be recognized by this microscopic observation in the testicle. He did not believe that the site of the carcinoma made any difference in the degree of the hyperplasia of the interstitial cells. These observations were in a measure corroborated by Ishiomoto,[2] who stated that hyperplasia of the interstitial cells is generally demonstrable in cases of carcinoma. However, he did not believe that this hyperplasia of the interstitial cells is different from that seen commonly in cases of tuberculosis. Beck [3] made a comparative study of the testicles from 30 patients with carcinoma and 35 persons who were carcinoma-free in the same age range. In none of the testicles studied did he find the changes which Sehrt had described as being characteristic of testicles from men dead of carcinoma.

From the comprehensive review of the literature by Rasmussen [4] one may judge that an apparent hyperplasia of the interstitial cells of

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1. Sehrt, E.: Centralbl. f. allg. Path. u. path. Anat. **54**:353, 1932.
2. Ishiomoto, Y.: Tr. Soc. path. jap. **24**:456, 1934.
3. Beck, von W.: Centralbl. f. allg. Path. u. path. Anat. **65**:65, 1936.
4. Rasmussen, A. T., in Cowdry, E. V.: Special Cytology, New York, Paul B. Hoeber, Inc., 1928, vol. 2, pp. 1209-1256.

the testicle is found associated with varying degrees of atrophy and fibrosis of the seminiferous tubules in a great variety of conditions. These conditions include the chronic ill health and malnutrition that accompany chronic tuberculosis, syphilis, cancer and pernicious anemia. The same alterations are present in cryptorchidism; in vasectomy or partial castration; in the testicular graft; in the displaced testicle; on changes in environmental temperature; on irradiation with roentgen rays and radium; in the reaction to healing of testicular wounds; on overeating; in inanition and dietary deficiency; on ingestion of drugs, and on administration of glandular extracts. The quantitative work of Slotopolsky and Schinz [5] indicated that the apparent hyperplasia of the interstitial cells is only relative owing to the tubular degeneration and atrophy. Houghton [6] studied the testicles from persons with mediastinal teratoma and pointed out the autonomous character of the tumor-like nodules of Leydig's cells, which do not adapt themselves to the existing structure of the tissue. Many of the seminiferous tubules are atrophic. However, because of the characteristic deposition of the interstitial cells he concluded that their increased prominence in the presence of mediastinal teratoma cannot be explained solely on the basis of a shift in relative values occasioned by the atrophic condition of the tubules.

Since the interstitial cells of the testicle secrete sex hormones important in the development of the secondary sexual characters, and since they have been shown to have a hormonal relationship with the pituitary gland, Sehrt's observation that they show a characteristic hyperplasia in the presence of carcinoma is considered as being worthy of verification. Therefore, this study is designed to determine whether there is a characteristic morphologic change in the interstitial cells of the testicles from patients with carcinoma of various parts of the body which can be distinguished by microscopic examination of a routine section of the testicle.

MATERIALS AND METHODS

Routine sections from the testicles of 173 carcinoma-free persons were stained with hematoxylin and eosin and examined microscopically to establish what might be termed standard interstitial cell contents. These persons ranged from 20 to 90 years of age. Testicles from patients whose condition had been diagnosed as tuberculosis or syphilis were excluded, for it is generally agreed that the interstitial cells may be increased in number in these conditions.

Each section was given a cursory general examination followed immediately by a careful count of the interstitial cells in five characteristic fields. It was found that all testicles could be divided into four groups on the basis of their interstitial cell contents. Testicles designated as 1 + contained groups of interstitial cells of 20 or less; those designated as 2 + contained groups of between 20 and 40

5. Slotopolsky, B., and Schinz, H. R.: Virchows Arch. f. path. Anat. **257**:294, 1925.

6. Houghton, J. D.: Am. J. Path. **12**:349, 1936.

interstitial cells; 3+ designated testicles which contained groups of from 40 to 60 interstitial cells; and testicles designated as 4+ contained groups of 60 or more cells.

The same criteria were applied in the determination of the interstitial cell contents of the testicles from 173 patients with carcinoma. This group included 84 patients with carcinoma of the gastro-intestinal system, 30 with primary carcinoma of the lungs, 20 with carcinoma of the genito-urinary system, 9 with carcinoma of the buccal cavity and pharynx, 8 with carcinoma of the skin, 9 with carcinoma of the pancreas, 3 with carcinoma of the liver, 2 with thyroid carcinoma, 1 with adrenal carcinoma and 7 with carcinoma of unknown origin.

To test the objectivity of the observations, sections of the testicles from 40 persons with carcinoma were mixed with those of 40 controls. The only selection within the group was on the basis of age, extreme care being observed to select the same number of control persons in each age decade as there were patients with carcinoma. The entire series of 80 testicles were given code numbers and then classified according to the interstitial cell contents.

The carcinoma-free group of persons (the controls) contained 30 persons with bronchopneumonia, 11 with lobar pneumonia, 39 with generalized arteriosclerosis, 12 with peritonitis, 21 with myocardial disease of various kinds, 5 with coronary thrombosis and infarction of the myocardium, 5 with pyelonephritis, 33 with chronic disease of various kinds, such as pneumonoconiosis, gastric and duodenal ulcers, and cirrhosis of the liver, and 13 with acute processes such as fractures, extensive burns and massive hemorrhage.

After the interstitial cell contents of the 346 testicles had been determined, all were grouped according to the disease, color and age of the subject.

As a further test of Sehrt's findings and to examine for the first time, so far as I know, the relation of the testicle to carcinoma in experimental animals, a study was made of the testicles of mice with transplanted mammary adenocarcinoma.

The mice were highly inbred males from 4 to 6 weeks of age.[7] This strain of mice has been made homozygous in all genetic factors by careful inbreeding since 1909 and has been used frequently in cancer research (Strong[8]). The closely inbred nature of the strain made it unnecessary to use litter mates. The males are susceptible to cancer transplantation but do not acquire tumors spontaneously.

The tumors are typical mouse tumors, that is, they are considerably softer than the cancers of the breast commonly found in human beings. They are friable, well vascularized and comprised largely of pale gray masses of epithelial cells. Frequently, areas of hemorrhage or necrosis are found. Microscopically, the tumor cells are large, polyhedral and occasionally show mitotic figures. In some situations they show the atypical acinar formation of adenocarcinoma; in other situations they show papilliferous and tubular arrangement, while in others they are disposed in masses typical of carcinoma simplex. Although no metastases are found, occasional ramifying masses of tumor cells are seen invading striated muscle.

Of the 132 mice used in the experiment, 72 were inoculated with tumors and 60 were retained for controls. All the mice were kept under uniform

7. The mice were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me. The tumor was sent through the kindness of Dr. C. C. Little and is known as dilute brown B (DbrB).

8. Strong, L.: J. Exper. Zoöl. **36**:78, 1922.

normal conditions and on a regular diet. This diet consisted of ground corn, wheat, whole milk powder, salt, wheat germ, yeast, Purina dog chow[9] and dried bread. Two cubes of the chow were supplied each week. The remainder of the diet was supplied ad libitum. Each tumor mouse was weighed once a week, at which time the growth of the tumor was determined by palpation. Careful note was made of the condition of the hair and the activity of the animal.

Most of the tumors were present at the end of two weeks. Many of the masses became cumbersome and were dragged over the floor of the cage.

Groups of tumor mice together with comparable groups of control animals were killed at various ages: group I, at from 9 to 11 weeks of age; group II, at from 11 to 13 weeks of age; group III, at from 12 to 14 weeks of age, and group IV, at from 14 to 16 weeks of age. Animals with large tumors were killed in each age group. Therefore, adolescent and young adult animals in which the tumors had been present five, seven, eight and ten weeks were studied together with comparable groups of tumor-free mice.

Each animal received a careful postmortem examination for evidence of metastasis. The tumor mass was then removed and weighed. Each tumor mouse stripped of its tumor and each control mouse was weighed. The tumors and bodies of the mice were fixed in a 40 per cent solution of formaldehyde diluted 1:10. After fixation testicles from 20 tumor mice and 20 controls were cleaned of epididymis, fat and other extraneous tissues and weighed. The tumors of the tumor mice and both testicles of all tumor and control animals were sectioned and stained with hematoxylin and eosin.

RESULTS

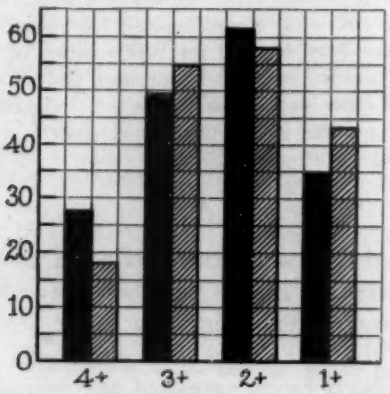
Seventy-six of the persons with carcinoma showed an interstitial cell content of the testicle of 3+ or more, but only 2 of the 76 had an interstitial cell content exceeding that of 72 of the controls of the same age group. In these 2 the interstitial cells were disposed in the form of large sheets far in excess of any individual collection seen in the control groups. Small groups of interstitial cells were found frequently in the inner margins of the tunica albuginea in testicles from both groups.

As a result of the objective classification of testicles from 40 patients with carcinoma and 40 carcinoma-free controls of similar age range there were 41 testicles with an interstitial cell content of 2+ or less and 39 with a content of 3+ or more. Of the 41 testicles with an interstitial cell content of 2+ or less, 20 were from patients with carcinoma, and 21 were from the controls; of the 39 testicles with an interstitial cell content of 3+ or more, 20 were from patients with carcinoma, and 19 were from the controls. There was no characteristic arrangement nor was there any degree of hyperplasia of the interstitial cells which served to identify the testicles as from patients with carcinoma or as from controls.

9. The chow is made up as follows: 5 per cent fat, 23 per cent protein, 4 per cent fiber, 7 per cent ash, 7 per cent moisture and 54 per cent nitrogen-free extract.

Classification according to the various systems involved by the tumors gave no noteworthy results. For example, one of the two testicles showing marked hyperplasia of the interstitial cells was from a patient with carcinoma of the prostate. However, the testicles from 9 other patients with prostatic carcinoma showed no significant hyperplasia or characteristic disposition of the interstitial cells. Classification of the testicles from the controls according to their disease processes gave no significant results.

The accompanying figure presents a comparison of the numbers of carcinomatous persons and of controls whose testicles were classified in respect to interstitial cell content as 4+, 3+, 2+ and 1+, irrespective of the age group, disease or site of the malignant growth. Of the 44 persons whose testicular classification was 4+, 27 had carcinoma



A comparison of the interstitial cell contents of testicles from 173 carcinomatous persons and 173 carcinoma-free controls. The black columns represent victims of carcinoma; the diagonally striped columns the carcinoma-free controls. The figures at the left represent the number of persons; those at the bottom, the interstitial cell contents.

and 17 were controls. In 2 of those who had carcinoma the interstitial cell content of the testicle was far in excess of that seen in any of the 17 used as controls. The fact that there are 10 more carcinomatous persons than controls with a classification of 4+ is not significant since nearly all patients with carcinoma die after more or less prolonged illnesses and with a great variety of concomitant and complicating diseases, especially generalized arteriosclerosis and pneumonia. Of the 17 controls with an interstitial cell content of 4+, 6 had pneumonia and 4 had generalized arteriosclerosis. The numbers of the carcinomatous subjects and controls with classifications of 3+, 2+ and 1+ are comparable.

Atrophy and fibrosis of the tubules were found in association with all large masses of interstitial cells in all age groups. Nevertheless there were persons in all decades whose testicles showed tubular atrophy and fibrosis without apparent hyperplasia of the interstitial cells.

There was no significant difference in the interstitial content of the testicles of white and colored persons whether they were victims of carcinoma or of various other diseases.

In general, the slight variation in interstitial cell content which occurred in various age decades was similar in both the carcinoma group and the carcinoma-free control group.

Examination of the testicles from mice with and without tumors disclosed no gross or microscopic difference. A comparison of the weights of 20 tumor mice and 20 control mice with the weights of their testicles revealed that the ratio between the weight of the testicle and the weight of the body of the tumor mouse stripped of the tumor was slightly less than that between the control testicle and the control mouse. However, no appreciable difference in ratio was found when the weight of the tumor was included in the calculation. There was no significant difference in the weights of the testicles from the 40 mice. No microscopic difference was observed. The various stages of spermatogenesis were present in all testicles. There was no tubular atrophy or fibrosis in any of the animal testicles.

COMMENT

Normal human material is difficult to obtain since most persons come to autopsy because of one or several diseases. The results in this study, therefore, are based on a comparison of testicles from persons dead of various diseases other than malignant disease, tuberculosis and syphilis with those from persons with carcinoma. It is realized that estimation of the interstitial cell content from a single section of a testicle probably gives a high percentage of error. However, the objectivity of the method of comparing a large number of sections of testicles made under similar conditions offsets to a great extent the possible error in the final results due to lack of application of more exact quantitative methods.

There is no evidence that the interstitial cells undergo a characteristic hyperplasia in a high percentage of cases of carcinoma as stated by Sehrt.[1] This investigation does indicate, however, that in some instances of carcinoma an apparent hyperplasia of the interstitial cells is associated with some degree of tubular atrophy and fibrosis, but this observation is in no way different from that in many other diseases.

The apparent hyperplasia of the interstitial cells was always associated with tubular atrophy and fibrosis, whereas the tubular changes frequently occurred in all age decades without there being any hyper-

plasia of Leydig's cells. Therefore, the negative findings in the animal testicles are significant since they showed no tubular changes and no apparent hyperplasia of the interstitial cells. These observations concur with the greater mass of evidence accumulated in the literature which indicates that an apparent hyperplasia of the interstitial cells when it occurs is always associated with tubular atrophy and fibrosis, but the converse is not always true. The tubular changes may be produced by a great variety of conditions, the most important of which are the malnutrition and chronic ill health associated with cancer and other chronic diseases.

SUMMARY AND CONCLUSIONS

There is a great "normal" variation in the interstitial cell content of the testicles.

There was no significant or characteristic hyperplasia of the interstitial cells in the testicles of 171 of the 173 carcinomatous persons when compared with those of an equal number of carcinoma-free controls from persons of similar age groups.

The application of quantitative methods when possible should clear up many of the discrepancies found in the reports in the literature on the state of the interstitial cells in various conditions.

In pathologic conditions in which there is apparent hyperplasia of the interstitial cells of Leydig this hyperplasia appears to be associated with atrophy and fibrosis of the tubules.

The apparent hyperplasia of the interstitial cells has essentially the same characters in all diseases which cause atrophy and fibrosis of the tubules.

Atrophy and fibrosis of the tubules may occur without there being any significant change in the number of interstitial cells.

CALCIFIC SCLEROSIS OF THE AORTIC VALVE (MÖNCKEBERG TYPE)

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The fact that fibrocalcific stenosis of the aortic valve is probably not always due to the same etiologic agent was recognized in 1846 by Hasse,[1] who attempted to differentiate atheromatous from endocarditic factors. However, the first systematic detailed study of this problem was reported in 1904 by Mönckeberg,[2] who described a type of aortic stenosis which he believed to be the result of primary sclerocalcific changes. Mönckeberg regarded the process as originating in small atheromatous plaques situated in the sinus pocket and believed that these plaques could increase in extent and ascend toward the free border of the valve. He further showed that the important feature differentiating this disease from the results of rheumatic fever lay in the fact that the essential sclerotic and calcific process is largely present in the fibrosa layer (Gross and Kugel [3]) of the aortic valve, whereas when it occurs as a secondary process in rheumatic disease it is confined chiefly to the ventricularis layer. Since the publication of this work the condition described by Mönckeberg has been termed "Mönckeberg's aortic sclerosis," "primary ascending sclerosis of the aortic valve" and "sclerosis annularis valvularum."

In contrast to Mönckeberg's primary ascending sclerosis, two chief types of "descending" sclerotic disease of the aortic valve are recognized. The first has already been referred to as the secondary calcific changes following rheumatic disease. This process affects the valve as a whole but tends to localize within the distal third of the leaflet. The second is the syphilitic type, in which the lesion extends from the base of the aorta through the commissures and passes horizontally around the free border of the cusps.

From the Laboratories of The Mount Sinai Hospital.

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1. Hasse, C. E.: An Anatomical Description of the Disease of the Organs of Circulation and Respiration, translated by W. E. Swaine, London, The New Sydenham Society, 1846, p. 134.

2. Mönckeberg, J. G.: Virchows Arch. f. path. Anat. **176**:472, 1904.

3. Gross, Louis, and Kugel, M. A.: Am. J. Path. **7**:445, 1931.

The reports dealing with the Mönckeberg form of sclerosis of the aortic valve represent two opposing views: On the one hand, Libman,[4] Ribbert[5] and Geerling[6] have considered the disease as purely degenerative, holding that inflammatory involvement either does not exist in connection with it or is secondary to it. On the other hand, Clawson,[7] Christian[8] and Anitschkow[9] have regarded all these aortic lesions as secondary to an inflammatory process, the former two workers holding that rheumatic disease is the etiologic agent. Margolis, Ziellessen and Barnes[10] studied forty-two hearts with calcific sclerosis of the aortic valve and could arrive at no definite conclusion concerning its pathogenesis, although they were inclined to the belief that a degenerative process probably plays a predominant rôle.

Microscopic study of such lesions invariably reveals an indolent process. There has been, however, no means available to determine with any degree of certainty whether the indolent process is the end-result of inflammatory disease or whether the mechanism is primarily degenerative. In recent years one of us (L. G.) with collaborators has demonstrated that rheumatic fever leaves in various sites in the heart, even in the heart in which the disease is completely extinct, certain stigmas which are definitely recognizable microscopically. Taken as a whole, these stigmas unmistakably stamp the site of the rheumatic process. In themselves they are for the most part nonspecific, and many of them are so insignificant that they must be sought for with great care. However, their persistent occurrence in a large proportion of rheumatic hearts, the presence of many of them in different sites and the existence of some of the more characteristic ones lend the greatest probability to a diagnosis of chronic or extinct rheumatic fever. These microscopic stigmas are found in the left auricle, the valve rings and valve leaflets, the intervalvular fibrosa, the conduction system, the aortic and pulmonic roots, the myocardium, the blood vessels and the pericardium.

The auricular stigmas (Gross[11]) include among others: mild lymphocytic infiltration of the endocardium; distortions and concentrations of

4. Libman, E.: M. Clin. North America **1**:573, 1917.

5. Ribbert, H., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1924, vol. 2, p. 195.

6. Geerling, J. G.: Sclerosis Annularis Valvularum, Thesis, University of Groningen, Holland, 1929.

7. Clawson, B. J.: Arch. Path. **12**:889, 1931.

8. Christian, H. A.: J. A. M. A. **97**:158, 1931.

9. Anitschkow, N., in Contributions to the Medical Sciences in Honor of Dr. Emanuel Libman by His Pupils, Friends and Colleagues, New York, International Press, 1932, vol. 1, p. 65.

10. Margolis, H. M.; Ziellessen, F. O., and Barnes, A. R.: Am. Heart J. **6**:349, 1931.

11. Gross, Louis: Am. J. Path. **11**:711, 1935.

the elastica; endocardial reduplications; increase and irregularity of endocardial smooth muscle; widening, fibrosis and increased vascularization of the subendocardium; marked hypertrophy and lymphocytic infiltrations of the auricular myocardium; fibrosis of the myocardium, and microscopic pericarditis. Of these lesions, endocardial reduplications, especially those of the multiple variety, hypervascularization of the subendocardium, penetration of capillaries into the endocardium, increase and irregularity of endocardial smooth muscle and microscopic pericarditis are highly suggestive of a rheumatic process.

Of considerable significance in this connection is the appearance of the valve rings. It has been shown by Gross and Kugel [3] that the normal aortic valve ring possesses no blood vessels and that they are rare in the mitral valve ring. When present, they consist of delicate capillaries of a characteristic configuration. Capillaries are present in a small percentage of the pulmonic and tricuspid valve rings. On the other hand, in over half the cases of inactive rheumatic fever studied by Gross and Friedberg [12] the subvalvular angle of the aortic valve ring showed involvement, that is, elastified reduplications, often multiple and sometimes vascularized. In most of the cases the ring showed scarring and occasionally rare lymphocytes and plasma cells. In a number of cases the ring contained capillaries, hyaline arterioles and hypertrophied vessels. In approximately 25 per cent of the cases all the valve rings were involved.

Examples of lesions of the valve leaflets (distal to the ring) which are highly suggestive of rheumatic damage are: vascularization, chiefly seen in the mitral and aortic valves (normal valves do not possess blood vessels [13]); widening, elastification and vascularization of the spongiosa layer [14]; thickening of the proximal layers of the valves (auricularis layer of the auriculoventricular valves and ventricularis layer of the semilunar valves),[14] and the formation of collagenous plaques at the closure line, which absorb the insertions of the chordae tendineae of the auriculoventricular valves and raise the semilunar folds of the semilunar valves (Gross and Friedberg [12]).

The intervalvular fibrosa [14] is normally nonvascularized and consists of dense collagen covered by the endocardial mantles. In 42 per cent of the cases in this series in which there was gross evidence of extinct polyvalvular rheumatic fever there were present elastification, mild lymphocytic infiltration and at times vascularization of the intervalvular

12. Gross, Louis, and Friedberg, C. K.: (*a*) Am. J. Path. **12**:469, 1936; (*b*) ibid., to be published.

13. Gross and Kugel.[3] Gross and Friedberg.[12b]

14. For definitions and descriptions of these cardiac sites see Gross and Kugel.[3]

fibrosa. Similar changes were found in the collagenous extensions of the septum fibrosum which surround the horizontal conduction system (Gross and Fried [15]).

Examples of lesions of extinct rheumatic disease in the aortic and pulmonic roots (Gross [16]) are: microscopic scars of several varieties; high incidence of capillarization; occasional intimal reduplications, and certain characteristic changes in the adventitia of the roots of large blood vessels.[17]

The myocardial rheumatic stigmas consist of: interfascicular scars, which lie between the muscle bundles; dense perivascular scars; occasional interstitial lymphocytes, and certain fibro-elastic changes in the blood vessels which resemble those found in nonrheumatic hearts except that the tempo of their formation is more rapid (Gross, Kugel and Epstein [17]).

Finally, some form of pericarditis is almost universally present in rheumatic fever, and microscopic evidence of pericardial irritation can be found invariably in various pericardial sites even in cases in which the process is totally extinct (Friedberg and Gross [18]).

In view of the ubiquity and consistency of the afore-mentioned changes at the sites of the extinct rheumatic process, it seems not unreasonable to suppose that if the Mönckeberg type of calcific aortic disease is based on an ancient rheumatic process, a diligent search for these lesions should reveal them in a representative proportion of the cases. On the other hand, the conspicuous absence or rarity of these lesions, together with the absence of other evidence of inflammatory disturbance, would strongly favor the view that the Mönckeberg process is primarily degenerative. With this in mind we examined eighteen cases of advanced calcific sclerosis of the aortic valve with and without stenosis which appeared to conform to the Mönckeberg type. It was soon found that in three of these cases there was unmistakable microscopic evidence of associated rheumatic disease in an extinct form, and that in four additional cases there was associated syphilis of the aorta involving the aortic commissures. There remained eleven cases of what appeared to be pure primary calcific disease of the aortic valve.

In order to compare the incidence of the lesions which might be present in this group with the incidence of the lesions in cases of rheumatic disease of such a type as might conceivably simulate the Mönckeberg process we also examined thirteen cases of grossly monovalvular extinct rheumatic disease. Also, the findings in nineteen cases of grossly polyvalvular extinct rheumatic disease are described in order to deter-

15. Gross, Louis, and Fried, B. M.: Am. J. Path. **12**:31, 1936.
16. Gross, Louis: Am. J. Path. **11**:631, 1935.
17. Gross, Louis; Kugel, M. A., and Epstein, E. Z.: Am. J. Path. **11**:253, 1935.
18. Friedberg, C. K., and Gross, Louis: Am. J. Path. **12**:183, 1936.

mine the incidence of these lesions in typical average rheumatic hearts free from active inflammation. Three additional cases were studied in order to determine the possible rôle which fibrous commissural bridging may play in the development of the Mönckeberg calcific disease of the aortic valve.

The technical methods employed in these studies were those previously described by Gross and Ehrlich.[19] Sections were cut according to the standardized method of Gross, Antopol and Sacks.[20] The most useful stains for the purpose of these studies were the hematoxylin and eosin, Weigert's elastic and Van Gieson's connective tissue stains. The clinical records were carefully studied for a history suggestive of rheumatic fever, syphilis or other infectious disease.

FINDINGS IN NINETEEN HEARTS SHOWING GROSSLY POLYVALVULAR EXTINCT RHEUMATIC DISEASE

The lesions listed in the table refer to those mentioned in foregoing paragraphs and were considered positive only when their nature and extent indicated that they were the result of an inflammatory process. As may be seen from the table, this group of hearts showed an extraordinarily high incidence of ring and leaflet lesions. Inasmuch as the percentage of incidence for each site named in the table is based on examinations of single representative sections from that site in the nineteen hearts, it is not unlikely, as has been previously indicated,[12] that serial sections might have revealed lesions in practically every valve ring. Various suggestive lesions of the types which have been mentioned were found in the left auricle in 69 per cent of the hearts, in the aortic root in 54 per cent and in the pulmonic root in 23 per cent. Of considerable importance was the finding of lesions in the intervalvular fibrosa in 42 per cent of the hearts. Pericardial lesions similar to those previously described [18] were present practically invariably in various cardiac sites.

FINDINGS IN THIRTEEN HEARTS SHOWING GROSSLY MONOVALVULAR EXTINCT RHEUMATIC DISEASE

As was mentioned, this group of hearts was selected in order to afford a closer comparison with the Mönckeberg series. As may be seen from the table, the incidence of mitral leaflet and ring lesions was almost exactly similar to that in the group with grossly polyvalvular extinct rheumatic disease. Even though the incidence of ring and leaflet lesions of the aortic, tricuspid and pulmonic valves was somewhat lower, respectively, the presence of such lesions in a large number of these hearts

19. Gross, Louis, and Ehrlich, J. C.: Am. J. Path. **10**:467 and 489, 1934.
20. Gross, Louis; Antopol, William, and Sacks, B.: Arch. Path. **10**:840, 1930

Comparison of Percentage Incidence of Microscopic Lesions of Various Sites in Hearts Showing Rheumatic Disease and Primary Calcific Disease of the Aortic Valve

Group	Mitral Valve, Posterior Cusp			Mitral Valve, Anterior Cusp			Tricuspid Valve			Pulmonary Valve			Aortic Valve			Left Auricle						
	Ring	Leaflet	Ring, Leaflet or Both	Ring	Leaflet	Ring, Leaflet or Both	Ring	Leaflet	Ring, Leaflet or Both	Ring	Leaflet	Ring, Leaflet or Both	Ring	Leaflet	Ring, Leaflet or Both	Endocardium	Myocardium	Pericardium	One or More Sites	Pulmonic Root	Aortic Root	Intervalvular Fibrosa
Mönckeberg series including hearts in which there was associated microscopic rheumatic or syphilitic disease (18).....	22	17	27	5	5	11	5	0	5	11	5	17	17	*	*	17	0	17	27	0	38	5
Mönckeberg series with hearts showing rheumatic disease eliminated (15).....	7	7	13	0	7	7	0	0	0	13	0	13	13	*	*	7	0	7	13	0	40	5
Uncomplicated Mönckeberg series, i. e., with hearts showing rheumatic and syphilitic disease eliminated (11).....	9	0	9	0	9	9	0	0	0	9	0	9	0	*	*	9	0	0	9	0	18	0
Hearts showing grossly monovalvular extinct rheumatic disease (13).....	47	74	89	53	95	95	32	26	47	26	5	26	58	21	58	63	37	74	95	37	68	5
Hearts showing grossly polyvalvular extinct rheumatic disease (19).....	84	84	92	54	92	100	69	54	92	61	15	61	77	77	82	61	15	54	69	23	54	42

* Mönckeberg process. No rheumatic stigmas.

indicates that strictly monovalvular rheumatic disease is unusual. This result was by no means unexpected and has been discussed in previous reports.[12] The presence of lesions in more than one valve by itself constitutes an important point in the differentiation of the findings in rheumatic disease from those to be reported in the Mönckeberg series. Auricular lesions were almost invariably present. In this connection it may be mentioned that lesions in the left auricle are one of the most important means of rapidly determining the presence of extinct or active rheumatic fever.[11] The incidence of aortic and pulmonic root lesions was similar to that in the grossly polyvalvular series. Of great interest was the low incidence of lesions of the intervalvular fibrosa. This was previously noted by Gross and Friedberg [12b] and was considered by them evidence that in mild rheumatic fever extension by contiguity through the intervalvular fibrosa occurred to a minimal extent. Microscopically the lesions of the valves in this group differed from those in the grossly polyvalvular series only in that the lesions in the relatively uninvolved leaflets were extremely mild.

FINDINGS IN EIGHTEEN HEARTS SHOWING MÖNCKEBERG'S CALCIFIC SCLEROSIS OF THE AORTIC VALVE

An examination of the observations in this group discloses a sharp contrast to the two groups already described. The significance of the observation of Mönckeberg's disease in association with rheumatic stigmas in three hearts and of its association with syphilis in four hearts will be taken up in the comment. With these hearts eliminated, this series reveals an extremely low incidence of lesions of the auricle, valve root, valve leaflet, aortic and pulmonic roots and intervalvular fibrosa, respectively. These scattered lesions can be accounted for chiefly by one heart in which most of them were present in a very mild form. It is not inconceivable that there were present in this heart completely healed inflammatory lesions, possibly of rheumatic nature, and that the primary sclerocalcific process in the aortic valve completely overshadowed the inflammatory phenomena due to the extinct rheumatic disease. Of particular importance is the complete absence of lesions in the left auricle and in the tricuspid valve, inasmuch as these two sites have been shown by Gross [11] and by Gross and Friedberg [12] to be involved more frequently in rheumatic disease than any other in the heart. In a few instances in this Mönckeberg series the aortic rings showed collections of capillaries, lymphocytes and plasma cells, but these were invariably in close proximity to a marked calcific process and were undoubtedly directly due to the latter. The intervalvular fibrosa was totally devoid of evidence of a previous inflammatory process. No Aschoff bodies (Gross and Ehrlich [19]), interfascicular scars or appreciable pericardial lesions were present.

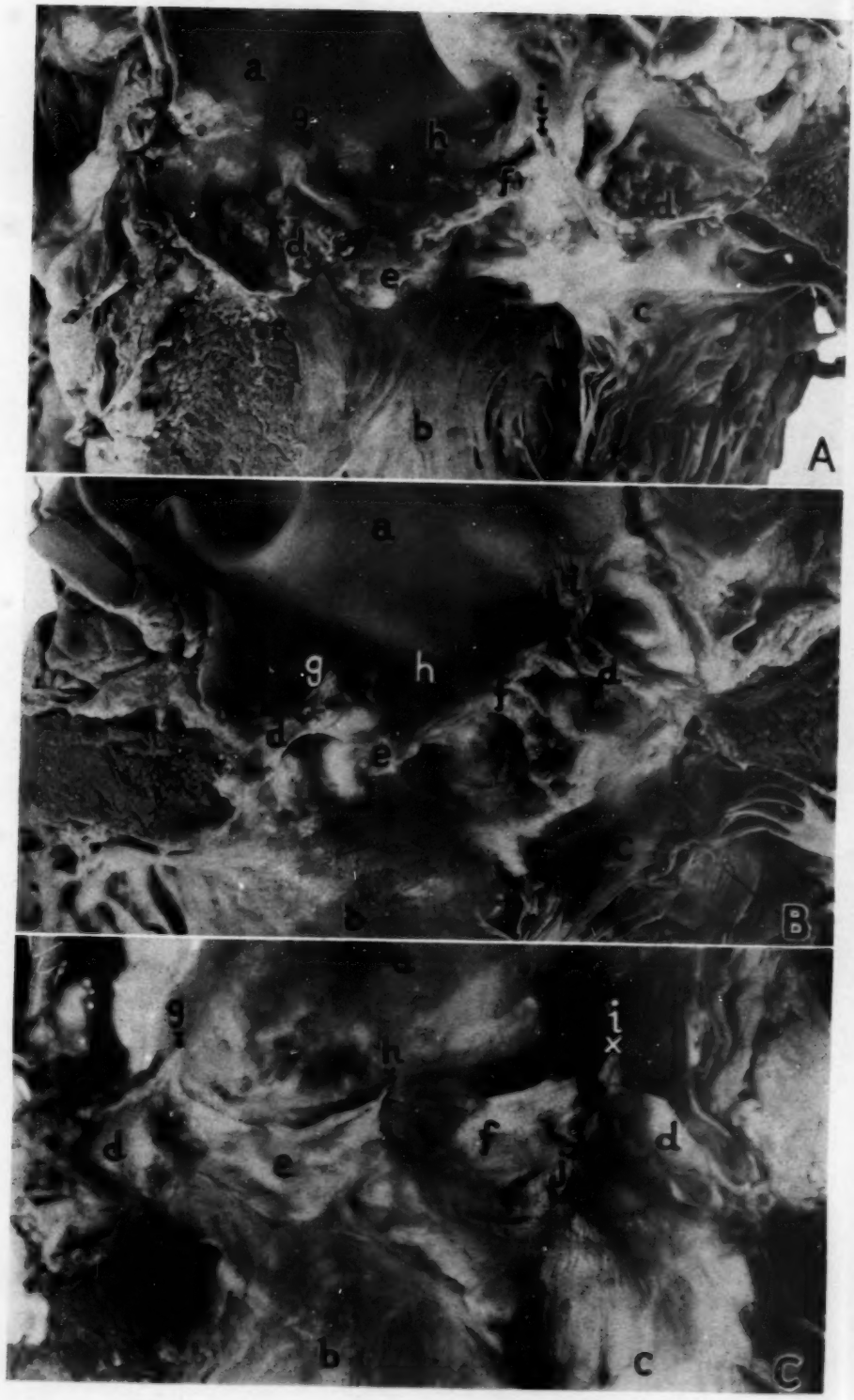

Figure 1

EXPLANATION OF FIGURE 1

Fig. 1.—*A*, gross photograph of the aortic valve of a patient aged 72 years in which uncomplicated calcific sclerosis of the Mönckeberg type produced marked stenosis: (*a*) aorta; (*b*) left ventricle, septal aspect; (*c*) anterior mitral cusp, ventricular aspect; (*d*) left aortic cusp, showing severe deformity due to projection of nodular calcific material toward sinus pocket; (*e*) right aortic cusp; (*f*) posterior aortic cusp; (*g*) left-right commissure; (*h*) right-posterior commissure, showing fusion and rounded calcific mass; (*i*) left-posterior commissure, showing fusion.

B, gross photograph of the aortic valve of a patient aged 62 years in which uncomplicated calcific sclerosis of the Mönckeberg type produced marked stenosis: (*a*) aorta; (*b*) left ventricle, septal aspect; (*c*) anterior mitral cusp, ventricular aspect; (*d*) left aortic cusp, showing marked thickening and deformity; (*e*) right aortic cusp, considerably shortened and deformed; (*f*) posterior aortic cusp, showing calcific nodules projecting through the ventricular surface; (*g*) left-right commissure, calcified, nodular, fused; (*h*) right-posterior commissure; (*i*) left-posterior commissure, fused.

C, gross photograph of an aortic valve in which are shown submarginal fibrotic commissural bridging and tension changes of the free edges of the cusps: (*a*) aorta; (*b*) left ventricle, septal aspect; (*c*) anterior mitral cusp, ventricular aspect; (*d*) left aortic cusp; (*e*) right aortic cusp; (*f*) posterior aortic cusp; (*g*) left-right commissure; (*h*) right-posterior commissure; (*i*) left-posterior commissure; (*j*) submarginal fibrous bridge agglutinating left and posterior cusps.

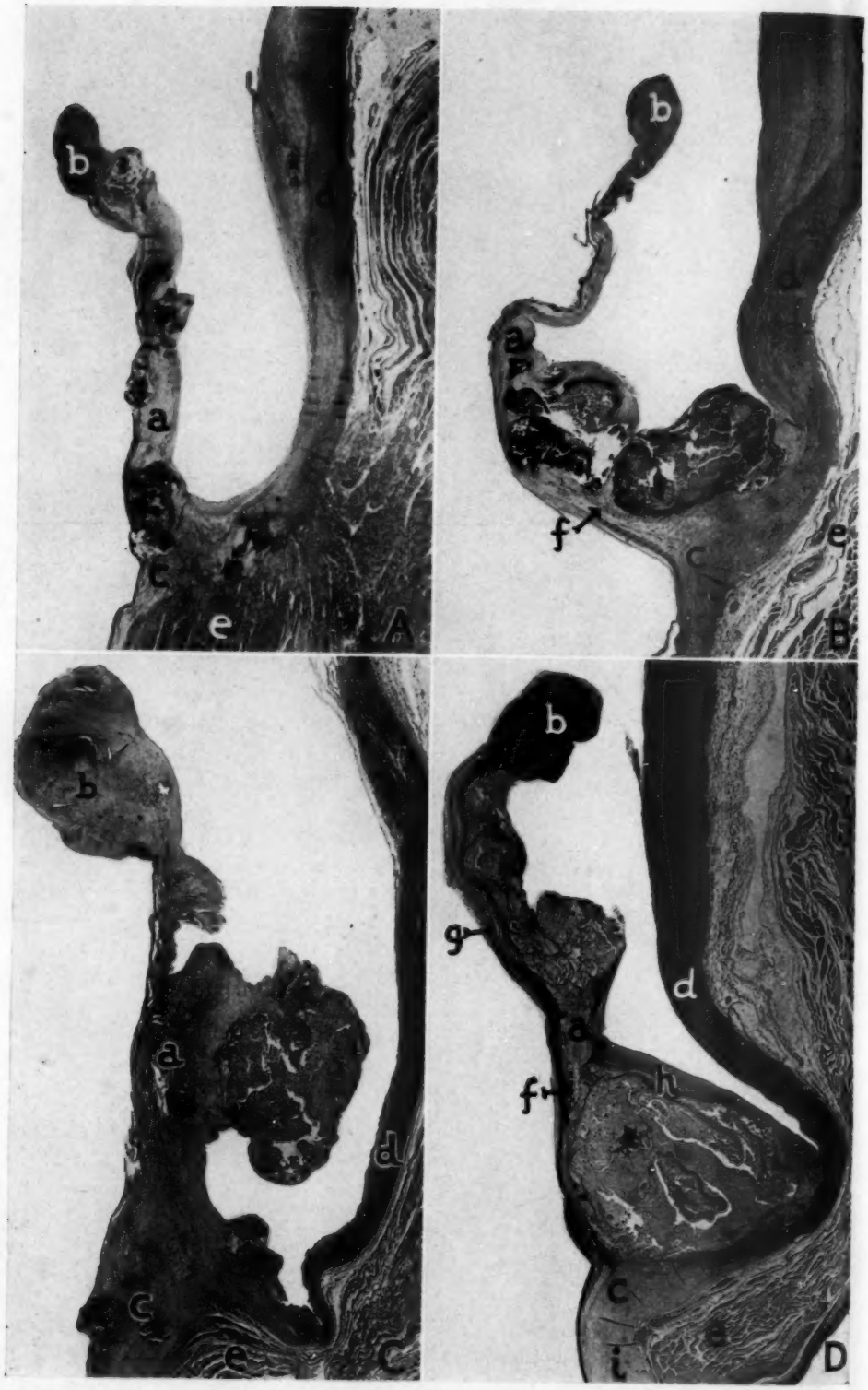

Figure 2

EXPLANATION OF FIGURE 2

Fig. 2.—*A*, cross-section of a cusp from the aortic valve of a patient aged 68 years, showing uncomplicated calcific sclerosis of the Mönckeberg type; low power magnification; hematoxylin and eosin stain: (*a*) fibrosa layer, showing small calcific masses entirely localized within it; (*b*) valve tip, consisting of the somewhat thickened ventricularis layer; (*c*) valve ring, intact; (*d*) root of the aorta; (*e*) myocardium.

B, cross-section of a cusp from the aortic valve of a patient aged 50, showing calcific sclerosis of the Mönckeberg type complicated by syphilitic aortitis; hematoxylin and eosin stain: (*a*) fibrosa layer, showing large calcific masses confined entirely to it; (*b*) valve tip, showing early thickening; (*c*) valve ring, intact; (*d*) root of aorta; (*e*) myocardium; (*f*) spongiosa layer, compressed but otherwise intact.

C, cross-section of a cusp from the aortic valve of a patient aged 62 years, showing uncomplicated calcific sclerosis of the Mönckeberg type; low power magnification; Weigert's elastic and Van Gieson's connective tissue stain: (*a*) fibrosa layer, showing masses of lime projecting through the arterialis layer toward the sinus pocket; (*b*) valve tip, considerably thickened, with collagenous whorls; (*c*) valve ring, containing small masses of lime; (*d*) root of aorta; (*e*) myocardium.

D, cross-section of a cusp from the aortic valve of a patient aged 72 years, showing uncomplicated calcific sclerosis of the Mönckeberg type; low power magnification; Weigert's elastic and Van Gieson's connective tissue stain: (*a*) fibrosa layer, almost completely replaced by calcific masses; (*b*) valve tip, consisting of the thickened ventricularis layer and showing whorl formation; (*c*) valve ring, intact; (*d*) root of aorta; (*e*) myocardium; (*f*) spongiosa layer, presenting a few vessels in proximity to calcific masses; (*g*) ventricularis layer, showing fibro-elastic thickening; (*h*) arterialis layer, showing fibrotic thickening; (*i*) intervalvular fibrosa, intact.

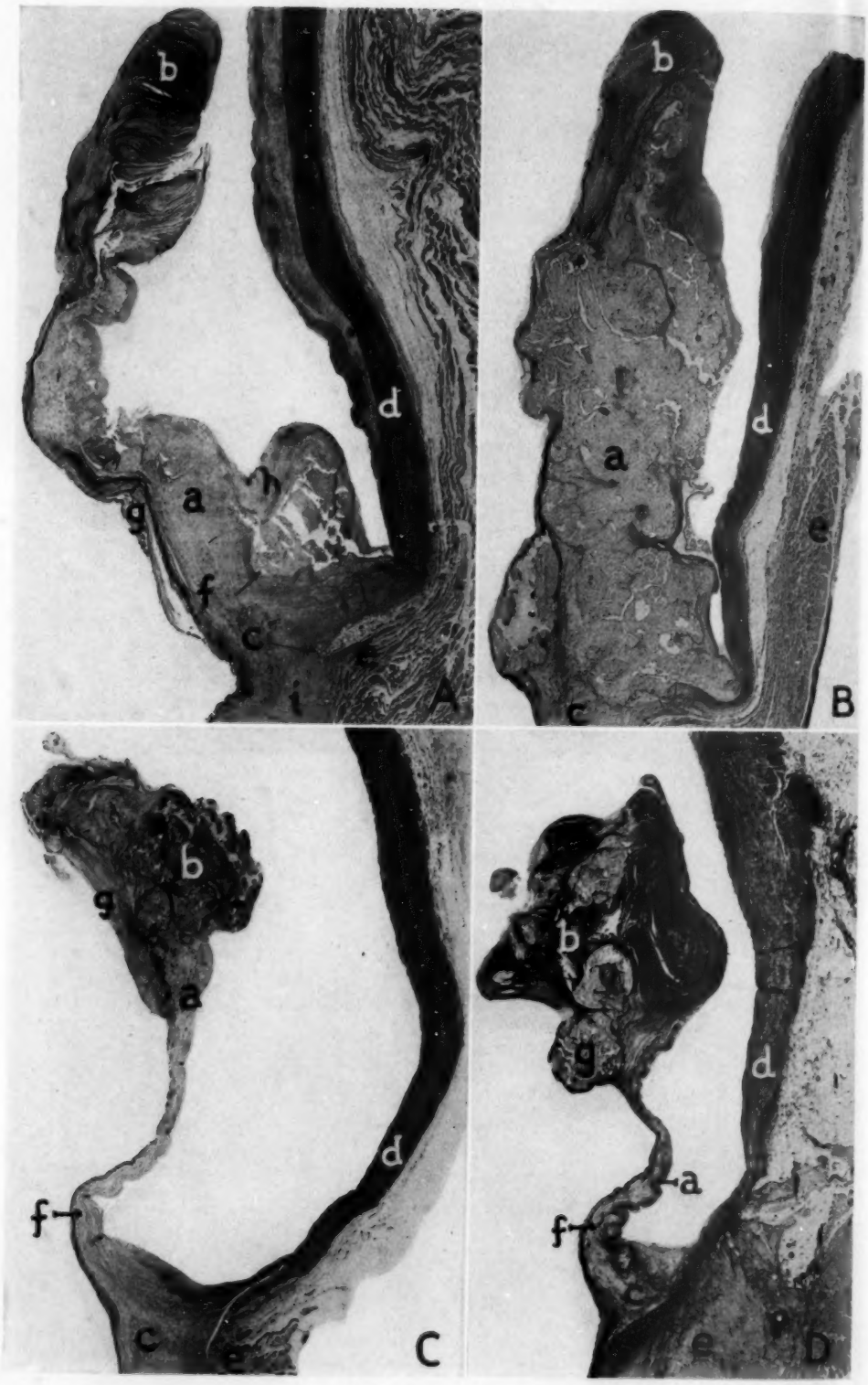

Figure 3

EXPLANATION OF FIGURE 3

Fig. 3.—*A*, cross-section of a cusp of the aortic valve from a patient aged 66 years, showing calcific sclerosis of the Mönckeberg type associated with syphilis of the aorta involving the commissures; low power magnification; Weigert's elastic and Van Gieson's connective tissue stain: (*a*) fibrosa layer, showing hyaline changes and infiltrated with lipoid and calcific masses; (*b*) valve tip, consisting of elastic collagenous whorls derived from the ventricularis layer; (*c*) valve ring, containing blood vessels; (*d*) root of aorta; (*e*) myocardium; (*f*) spongiosa layer, completely compressed; (*g*) ventricularis layer, showing fibroelastic thickening; (*h*) arterialis layer, showing fibrotic thickening; (*i*) intervalvular fibrosa, intact.

B, cross-section of a cusp of the aortic valve from a patient aged 62 years, showing uncomplicated calcific sclerosis of the Mönckeberg type; low power magnification; Weigert's elastic and Van Gieson's connective tissue stain: (*a*) fibrosa layer, entirely replaced by enormous masses of calcific material; (*b*) valve tip, consisting of the thickened ventricularis layer; (*c*) valve ring, intact; (*d*) root of aorta; (*e*) myocardium.

C, cross-section of a cusp of the aortic valve from a patient aged 45 years, showing uncomplicated calcific sclerosis of the Mönckeberg type; low power magnification; Weigert's elastic and Van Gieson's connective tissue stain: (*a*) fibrosa layer, intact; (*b*) valve tip, markedly thickened by large calcific masses which almost entirely replace the fibrosa layer; (*c*) valve ring, intact; (*d*) root of aorta; (*e*) myocardium; (*f*) spongiosa layer, intact; (*g*) ventricularis layer at tip of valve, thickened.

D, cross-section of a cusp of the aortic valve from a patient aged 49 years, showing extinct rheumatic disease with secondary calcification; low power magnification; Weigert's elastic and Van Gieson's connective tissue stain: (*a*) fibrosa layer, intact; (*b*) valve tip, markedly thickened and consisting of whorled elastic collagenous transformation of spongiosa and ventricularis layers containing calcific masses; (*c*) spongiosa of the valve ring, widened and containing lymphocytes and capillaries; (*d*) root of aorta; (*e*) myocardium; (*f*) spongiosa layer, widened and containing capillaries; (*g*) calcific mass within the ventricularis layer.

In contrast to the marked tendency for the secondary calcific phenomena in rheumatic valvulitis to occur in the spongiosa and ventricularis layers of the aortic valve (fig. 3 *D*) such alterations were chiefly found in the fibrosa layer in the Mönckeberg series (fig. 2 *A, B* and *D*; fig. 3 *A, B* and *C*). Only when the process was extremely marked and irregular did these calcific nodules penetrate through the enveloping layers (fig. 2 *C*). In spite of the presence of commissural agglutinations in these cases, inflammatory phenomena were at a minimum. The mechanism concerned in the formation of the commissural agglutinations will be considered in the discussion.

The gross appearance of the Mönckeberg calcific aortic stenosis is too well known to merit elaborate discussion. Suffice it to state that in the typical form the valves are transformed into stiffened irregular nodular leaves, with most of the thickening taking place throughout the body of the leaflet rather than at the free edge (fig. 1 *A* and *B*). In advanced stages secondary sclerotic transformation produces extraordinary and bizarre deformities. The nodules vary considerably in their extent, are extremely hard and rounded or sharply irregular, and frequently penetrate through the ventricularis and arterialis enveloping layers of the cusp. The commissural agglutinations may be quite sharp in the milder forms. On the other hand, in advanced stages they may be broadened, nodular and distorted and show considerable irregular deformities. They differ in this respect from the evenly rounded and broadened lesions often seen in subacute bacterial endocarditis as well from the delicate grooved agglutinations found in the pure rheumatic process. The edges of the aortic leaflets in the Mönckeberg deformity may be sharp or thickened and distorted. They generally do not present the rolled and inverted gross configuration characteristic of the rheumatic lesion. It must be mentioned, however, that particularly after severe fibrotic changes have set in it may be difficult or impossible to differentiate the valve with the Mönckeberg lesion from the secondary calcific rheumatic valve.

Histologically, the Mönckeberg lesion can be easily distinguished from that found in rheumatic fever. The earliest changes occur in the fibrosa layer, generally near the base of the leaflet. Subsequent to sclerotic and hyaline transformation of the collagen within the fibrosa layer, lipoid changes appear, and with these there is soon deposited globular, amorphous or crystalline calcific material (figs. 2 *A* and 3 *A*). The nuclei in the fibrosa collagen tend to disappear. The calcific masses coalesce, and in their proximity lymphocytic infiltrations may be seen at times. When the process becomes very marked there occasionally takes place bony transformation, bone marrow metaplasia and the development of capillaries, muscular vessels, lymphocytes and plasma cells. Simultaneously, particularly if the process is situated close to the ring,

capillarization of the latter with mild lymphocytic infiltration may occur. Early in the process the calcific plaques are generally confined to the fibrosa layer. However, with continued evolution of the calcific process the spicules may penetrate into the ventricularis and arterialis layers (fig. 2 *C*). Under these conditions the enveloping layers become thickened and collagenous and ruptures of the elastica may take place. Depending on the site from which the section is cut, there may be a preponderance of the calcific process at the base, middle or tip of the leaflet (fig. 2 *B* and *C*; fig. 3 *C*). Occasionally nodules seem to originate from the edges of the fibrosa layer and spread through one or the other of the enveloping layers (ventricularis or arterialis), forming polypoid masses (fig. 2 *C*). Apart from these primary calcific deposits in the fibrosa layer, similar deposits on a considerably smaller scale may be occasionally encountered apparently as a primary process in the ventricularis layer. The otherwise intact spongiosa layer is usually squeezed and narrowed but often remains distinctly discernible. Owing to stiffening and deformity of the valve the tip frequently develops bulbous elastic collagenous whorls similar to those resulting from tension changes without deposition of calcium salt (fig. 2 *B, C* and *D;* fig. 3*A*). Blood vessels of the rheumatic type are never seen in the valve leaflets or rings.

In contrast to this, the rheumatic calcified valve presents marked thickening and often vascularization of the spongiosa and ventricularis layers, in which deposition of lime also takes place. At times the lime extends into the fibrosa layer. Apart from this, the fibrosa may contain small spicules of lime due to a simultaneous degenerative process. Lesions of the valve ring are almost invariably present (fig. 3 *D*), as are vascularization of the spongiosa and thickening of the valve with entropion of the free edge.

FINDINGS IN THREE HEARTS SHOWING COMMISSURAL BRIDGING OF NONINFLAMMATORY NATURE

We have studied the hearts of three patients past middle age with negative clinical histories in whom the only significant pathologic cardiac changes were the development of commissural submarginal agglutinations in the aortic valve (fig. 1 *C*) and tension changes at the free edges of the cusps. The complete absence of vascular or exudative phenomena in these patients renders it extremely unlikely that these agglutinations were on an inflammatory basis. Various grades of degenerative lesions and fibroblastic proliferation were noted at the commissural region, which indicated that such agglutination and bridging might be due to primary degenerative with possibly secondary local inflammatory processes. The consequent stiffening of the cusps together

with the mild stenosis could conceivably produce a valvular deformity of sufficient extent to submit the leaflets to unusual stress. As in other parts of the cardiovascular system, tissues under these hydrodynamic conditions are apparently subject to secondary degenerative changes of the collagen with subsequent lipoid and lime infiltration. The finding of calcified nodules within the fibrosa layer at the base of the valve in two of these hearts, as well as the similarity of these nodules to those seen in the valve with the Mönckeberg process, lends additional evidence to the view that such sclerotic commissural bridging may play a rôle in at least some cases of calcific sclerosis of the aortic valve.

COMMENT

The eighteen hearts comprising the Mönckeberg series described in this report can be divided into two groups, viz., those in which the Mönckeberg deformities were associated with rheumatic disease or syphilis (seven hearts) and those in which the Mönckeberg process was uncomplicated (eleven hearts). The presence, consistently, of a great variety of lesions in the hearts with the completely healed rheumatic process, even when this was of so mild an extent that it was recognized grossly as monovalvular, affords a sharp contrast to the almost complete absence of these lesions from the eleven hearts with uncomplicated Mönckeberg's calcific aortic stenosis. Even if the three hearts showing coincidental rheumatic disease and the four showing coincidental syphilis of the aortic valve are merged with the uncomplicated Mönckeberg group, the incidence of these various lesions is still extraordinarily low in the latter as compared with the rheumatic group (see table).

Three interpretations of the observations, particularly those in the uncomplicated Mönckeberg group, merit consideration. One is that in spite of the scarcity or absence of stigmas implicating rheumatic fever as the etiologic agent, this disease is nevertheless responsible for the Mönckeberg process. Against this assumption is the fact that even in cases in which there is no positive or only a suggestive clinical history and in which the rheumatic fever has been of the mildest form and is completely healed, the diagnosis of which rests entirely on the presence of relatively and individually insignificant changes (as exemplified by the grossly monovalvular group) the presence of the aforementioned stigmas is nevertheless consistent and widespread. Occasionally they are highly characteristic and even pathognomonic of the disease. Their scarcity or absence from the uncomplicated Mönckeberg group, together with the clearcut histologic differences from the rheumatic calcific process, indicates that unless further evidence can be produced to the contrary it is extremely unlikely that rheumatic fever is responsible for the Mönckeberg process.

The view that some nonspecific inflammatory change initiates the calcific degenerative phenomena is equally lacking in direct evidence to support it. The only inflammatory changes which are occasionally found are those directly due to the presence of irritating lipoid and calcific spicules. These are in direct contiguity with the mild inflammatory change. Furthermore, in most of the Mönckeberg series no evidence of inflammatory lesions was shown even in the neighborhood of the calcific plaques. Finally, as has been indicated, early stages in the Mönckeberg sclerosis fail to disclose a primary inflammatory basis.

The most likely conclusion based on our present findings, therefore, is that this disease is primarily degenerative. Whether early degenerative agglutinations of the commissures, producing additional stress and strain on the leaflets, lead to continued hyalinization of the fibrosa collagen with subsequent lipoid and calcific changes, or whether these occur ab initio without such preliminary deformity of the valvular mechanism, being due possibly to mechanical factors of stress and strain, it would be rash to decide. As is well known, in the later age periods such hyalinization of the mitral valve collagen, followed by lipoid and calcific changes, is not infrequently encountered. This occurs quite independently of the presence of inflammatory changes in the valve and may produce lesions identical histologically with those found in the Mönckeberg process. Furthermore, the age period at which this process may take place varies considerably with the patient. Thus, the changes may be absent in the fifth and sixth decades and they may be present in the third decade. This individual predisposition to such degenerative and infiltrative processes has been discussed in a previous publication and is in keeping with the observations of Libman,[21] who has called attention to a diathesis which may exist in certain persons toward their formation.

In the light of the foregoing discussion the coexistence of inflammatory disease in seven of the eighteen hearts with Mönckeberg's aortic calcific disease, otherwise identical grossly and histologically with the primary group, assumes special significance. With the demonstration that the Mönckeberg process is primarily and purely a degenerative lesion, the high incidence of associated inflammatory disease in the Mönckeberg series suggests a causal relation. The explanation which appears most acceptable is that such primary inflammatory damage to the aortic valve (or mitral and aortic valves in rheumatic fever) probably imposes additional factors of mechanical strain which are followed in susceptible persons by deposition of lipoid and lime in the fibrosa layer—a process which eventually assumes the proportion of Mönckeberg's disease. Put in other words, under suitable conditions there

21. Libman, Emanuel: Tr. A. Am. Physicians **43**:188, 1928.

occurs, chiefly in the aortic valve, a series of degenerative lipoid-depositing and calcific processes which are recognizable as Mönckeberg's aortic calcific disease. In some persons in whom there is a propensity toward the initiation and continuation of these degenerative processes the ordinary wear and tear imposed on the aortic valve are sufficient to lead to marked calcification of the structure. In others this degenerative process sets in only after inflammatory damage has rendered the valve additionally susceptible to mechanical strain. In most persons, however, the propensity toward the calcific process in the aortic valve is insufficient to permit this process to continue to an appreciable extent even in the presence of considerable valvular deformity.

SUMMARY

There have been described in this report the findings in eighteen hearts with so-called Mönckeberg's calcific sclerosis of the aortic valve, in nineteen hearts with a grossly polyvalvular extinct rheumatic process and in thirteen hearts with a grossly monovalvular extinct rheumatic process. Attention is drawn to the essentially different gross and microscopic features of the Mönckeberg and rheumatic valvular lesions. It has been shown that the heart with the uncomplicated Mönckeberg process shows practically none of the stigmas of extinct rheumatic fever and no other evidence which would indicate that the process is secondary to inflammatory changes. A discussion is given of the possible mechanisms concerned in the development of the essential Mönckeberg process, from which it appears that this is purely and primarily degenerative, its occurrence and extent depending in all probability on individual predisposition to collagen involution and lipoid and calcium deposition. The findings in three hearts with submarginal aortic commissural bridging of noninflammatory nature suggest that stress and strain in the aortic valve may serve as additional factors predisposing to degenerative processes. It is suggested that in certain persons in whom there exists a predisposition toward the deposition of lipoid and calcium, inflammatory lesions with subsequent deformity of the aortic valve may impose sufficient strain on the valve to initiate the Mönckeberg process.

HISTOLOGIC FEATURES OF THE INTRADERMIC REACTION TO TUBERCULIN IN CATTLE

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In 1908 Moussu and Mantoux [1] recommended the intradermic injection of tuberculin as a means of diagnosing tuberculous infections in cattle, and since that time this procedure has received wide acceptance. At present the intradermic test has superseded all others as a means of disclosing tuberculous infections not only in cattle but in other domesticated species of animals as well. As an illustration of the extensive use of this test it is of interest to note that in the United States during the fiscal year ending June 30, 1935, more than twenty-five million cattle were tested by this method in the furtherance of the cooperative state and federal program for the eradication of tuberculosis.[2]

It is not our intention in this report to consider the clinical aspects of the intradermic test or to evaluate the specificity of the procedure. This has been competently done by Römer and Joseph,[3] Haring [4] and others. There remains, however, one aspect of the intradermic test which, so far as we are aware, has not heretofore been studied. We refer to the local histologic changes that follow the intradermic injection of tuberculin into spontaneously sensitized cattle. The phenomenon has been studied experimentally in guinea-pigs by several workers, including Auché and Augistrou,[5] Spehl,[6] Dienes and Mallory [7] and Laporte,[8] and it seems incredible that no report is available concerning the histologic changes which the reaction produces in cattle. In order

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1. Moussu, G., and Mantoux, C.: Compt. rend. Acad. d. sc. **147**:502-504, 1908.
2. Report of the Special Committee on Tuberculosis, J. Am. Vet. M. A. **87**: 468-470, 1935.
3. Römer, P. H., and Joseph, Karl: Beitr. z. Klin. d. Tuberk. **14**:1-35, 1909.
4. Haring, C. M.: Proc. Am. Vet. M. A., 1910, pp. 306-314.
5. Auché, B., and Augistrou: Compt. rend. Soc. de biol. **68**:330-332, 1910.
6. Spehl, Paul: Arch. de méd. expér. et d'anat. path. **25**:239-269, 1913.
7. Dienes, Louis, and Mallory, T. B.: Am. J. Path. **8**:689-709, 1932.
8. Laporte, R.: Compt. rend. Soc. de biol. **114**:991-994, 1933.

to obtain information on the nature of the local histologic response we made the observations to be reported in this communication.

The information which we hoped to obtain included (1) the character of the local alterations in tissues and the cellular response in cattle considered clinically as having shown a positive relation to tuberculin administered intradermally and (2) the correlation, if any, between the character of the histologic reaction and the extent or severity of tuberculous infection as ascertained at necropsy.

MATERIAL STUDIED

The material which was used for this study was obtained from two groups of animals.

GROUP 1.—This group, which may be termed "the experimental group," consisted of four apparently normal calves, approximately 2 months of age. These calves were obtained for the purpose of studying the histologic changes of the intradermic tuberculin reaction in bovine animals sensitized experimentally. Two of the animals were given subcutaneous injections of 2 cc. of bovine tubercle bacilli suspended in saline solution,[9] and one calf received a similar injection of tubercle bacilli of avian origin. The fourth animal was not given an injection of tubercle bacilli, being utilized as a control. The animals were kept in separate stalls, and after the lapse of sixty days each received into the tissues of the left caudal fold an intradermic diagnostic dose (0.1 cc.) of mammalian tuberculin. Into the right caudal fold 0.1 cc. of avian tuberculin[10] was injected intradermally at the same time. Seventy-two hours after the injection of the tuberculin, portions of the skin from the test areas were excised under local anesthesia and preserved in a dilute solution of formaldehyde U. S. P. (1:10) for subsequent study.

GROUP 2.—This group, which may be termed "the clinical group," consisted of sixty-six head of adult cattle which were members of thirty-three separate farm herds. These herds had been tested with tuberculin in Minnesota during the furtherance of the cooperative state and federal program for the eradication of tuberculosis. All the herds had been tested from one to several times previously, and tuberculous infection was known to have existed in the majority of the animals of the respective herds prior to the last test. The usual dose of mammalian tuberculin was administered intradermally into the tissues of one caudal fold of each animal of the respective herds, and from some animals in which a positive reaction occurred tissues were removed for study. The tissues were excised at the time the results of the tuberculin tests were recorded. In the majority of instances this was approximately seventy-two hours after the tuberculin had been injected.

METHODS

The excised tissues were placed in a dilute solution of formaldehyde. After fixation, two or more blocks of each specimen of tissue usually were embedded in paraffin and cut into sections. Sections from all the specimens were stained with

9. The organism had been isolated three months previously from a spontaneously infected lymph node of a cow. The bacterial suspension had a turbidity comparable to tube 1 of the McFarland nephelometer.

10. All the tuberculin used was supplied by the Bureau of Animal Industry, United States Department of Agriculture.

hematoxylin and eosin; in addition, special stains, such as the Mallory-Heidenhain stain, Van Gieson's stain and Pap's [11] reticulum stain, were used to demonstrate certain features.

GROSS MORBID ANATOMY

After the tissues which were the site of the local tuberculin reaction had been excised, the animals were slaughtered and subjected to careful postmortem examination for lesions of tuberculosis.

GROUP 1.—Seventy-two hours after the administration of tuberculin the local reactions of the calves in group 1 were as follows: calf 1, which had been sensitized with bovine tubercle bacilli, showed a P 10 reaction to mammalian tuberculin and a P 3 reaction to avian tuberculin (fig. 1 *A* and *C*); [12] calf 2, which had been sensitized with bovine tubercle bacilli, showed a P 10 reaction to mammalian tuberculin and a P 2 reaction to avian tuberculin; calf 3, which had been sensitized with avian tubercle bacilli, showed a P 2 reaction to mammalian tuberculin and a P 4 reaction to avian tuberculin (fig. 1 *B*); calf 4, which had not been sensitized experimentally, did not reveal any gross evidence of reaction to either mammalian or avian tuberculin.

Results Following the Intracutaneous Injection of Tuberculin into Three Experimentally Infected Calves *

Calf	Type of Infection	Degree of Reaction to Tuberculin After Seventy-Two Hours		Lesions of Tuberculosis
		Mammalian	Avian	
1	Bovine	P 10	P 3	Involvement of cubital, prescapular and inferior cervical lymph nodes; progressive lesions
2	Bovine	P 10	P 2	Involvement of cubital, axillary, inferior cervical, prescapular, bronchial, mediastinal and portal lymph nodes; lesions of lungs
3	Avian	P 2	P 4	No demonstrable lesions

* The fourth calf in group 1, which was not infected experimentally, did not react to mammalian or to avian tuberculin, and necropsy did not reveal any tuberculous lesions.

Definite and characteristic lesions of tuberculosis were present in both calves which had received injections of bovine tubercle bacilli, but no demonstrable lesions were observed in the calf which had received the avian tubercle bacilli. Likewise, tuberculous lesions were not present

11. Pap, Tibor: Centralbl. f. allg. Path. u. path. Anat. **47**:116-117, 1929.

12. The code used to designate the local tuberculin reactions is that adopted by the United States Livestock Sanitary Association and approved by the Bureau of Animal Industry of the United States Department of Agriculture. P 1 indicates a circumscribed swelling in an area three-sixteenths inch (0.5 cm.) in diameter. Larger nodular reactions are recorded as P 2, P 3, P 4 and so forth. If the swellings are diffuse rather than nodular, the phrase "thick 2 ×" is the basic standard. This signifies that the caudal fold is twice the normal thickness. Larger diffuse swellings are recorded as "thick 3 ×," "thick 4 ×" and so forth.

in the calf which had not received an injection of tubercle bacilli. The results of necropsy on the four calves in group 1 are summarized in the accompanying table.

GROUP 2.—The listing of the degrees of the local reactions in each of the sixty-six animals in which a positive tuberculin reaction occurred seems unnecessary. Suffice it to say that the reactions recorded varied from the P 1 reaction to those considered as P 6. That there was no significant correlation between the severity of the local reaction and the amount of tuberculosis present in the respective animals was apparent from the fact that two of the carcasses that were condemned on account of extensive tuberculosis were those of animals in which the tuberculin reaction was recorded as P 1, while in several of the animals which did not have demonstrable lesions of tuberculosis the tuberculin reactions were recorded as P 3 or P 4.

All animals in this group were slaughtered under federal supervision; necropsy disclosed lesions of tuberculosis in forty of the carcasses, and in nine instances the disease was so extensive as to necessitate condemnation of the entire carcass. In four animals the only abnormality was the so-called "skin lesion," or subcutaneous tuberculoid lesion.[13] In twenty-one, or 31.7 per cent, of the carcasses no gross evidence of tuberculosis could be found. No postmortem data were available on one of the animals.

HISTOLOGIC PICTURE OF THE LOCAL TUBERCULIN REACTION

GROUP 1.—The histologic picture of the reaction following the intracutaneous injection of mammalian and avian tuberculin was essentially the same in each of the three calves which had previously received injections of virulent tubercle bacilli. Although the character of the elements entering into the reactive process was not dissimilar in the respective animals, there apparently was a marked difference in the degree or intensity of the reaction. The homologous tuberculin in each instance induced a much greater inflammatory response than did the heterologous tuberculin in the same animal (fig. 1 *A* and *B*). The marked or severe type differed from the mild or moderate type of reaction by an excessive amount of edema and the tendency of the tissues to undergo necrobiosis. It was also observed that a greater degree of sensitivity as determined by reactions to homologous tuberculin had developed in the animals which had received bovine tubercle bacilli than in the calf which had received avian organisms.

Microscopically, the most constant feature of the histologic picture was the occurrence of variable numbers of cells, predominantly histiocytic, which showed definite and characteristic predilection for the adventitial zone of the blood vessels and the tissues of the epineurium (fig. 2).

13. Feldman, W. H.: Arch. Path. **17**:533-545, 1934.

While the majority of these cells were histiocytic, few or a moderate number of lymphocytes were usually present. Polymorphonuclear leukocytes were rarely observed, although mononuclear and polynuclear acidophilic granulocytes were seen in variable numbers in the different reactions studied. In the severe reactions to homologous tuberculin the foci of reacting cells were distributed throughout the papillary and reticular layers of the derma and in the subcutaneous tissue (fig. 3).[14]

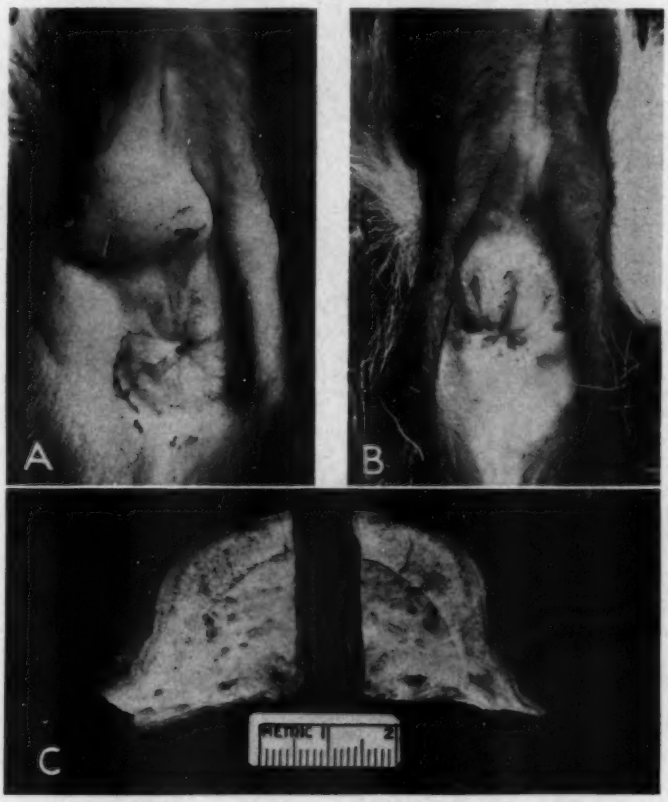

Fig. 1.—*A*, the local reaction which occurred seventy-two hours after the injection of mammalian and avian tuberculin into the caudal folds of calf 1 (sensitized with bovine tubercle bacilli). Mammalian tuberculin provoked a greater reaction. *B*, the reaction which followed the injection of mammalian and avian tuberculin into the caudal folds of calf 3 (sensitized with avian tubercle bacilli). The left caudal fold, into which avian tuberculin was injected, showed a more marked reaction. *C*, a transverse section of the caudal fold of calf 1, showing the reaction which occurred seventy-two hours after the injection of mammalian tuberculin; the upper limits of the edematous region are sharply demarcated.

14. The papillary and reticular zones of the derma of cattle are indistinctly demarcated, and many of the tissue elements of the reticular zone are continuous with those of the subcutaneous tissue.

If the reaction was less severe the cellular accumulations were most abundant in the reticular zone of the derma. In many instances in which the perivascular reaction was particularly vigorous the proliferating histiocytic cells had encroached on the small vascular channels to such an extent as to cause the lumen to disappear. Giant cells of the Langhans type could occasionally be seen among the reactive foci, and it was not difficult to demonstrate mitotic division among the histiocytic cells (fig. 4).

Edema was a variable feature, being present in slight degree or even absent in the reactions of mild severity but constituting a prominent

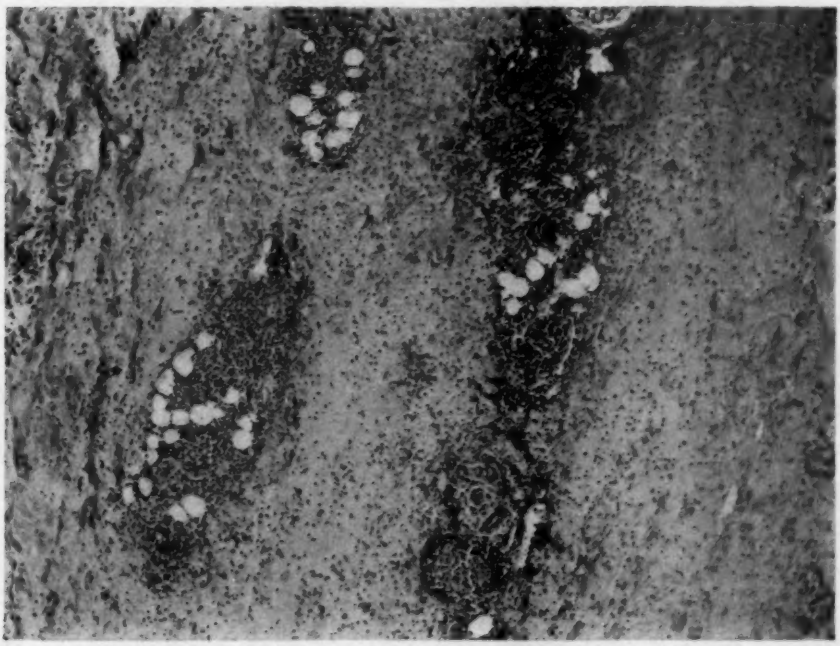

Fig. 2.—Large collection of histiocytes and considerable edema in the reticular zone of the derma of calf 2; × 65. A few spaces that were formerly occupied by fat are present.

part of the lesion in those instances in which the reactive process was energetic and severe. In severe reactions the edematous condition frequently extended from the malphigian layer of the epidermis through the subcutaneous layer. Wandering cells or macrophages in moderate number were commonly observed in the exudative fluid. Thrombi were infrequently seen occupying the lumen of some of the smaller vessels, but endovascular changes of a progressive character were not seen. Signs of beginning necrobiosis were detectable by the occurrence of pyknosis among many of the cells of certain of the perivascular areas of reaction, but extensive or advanced necrosis had not occurred.

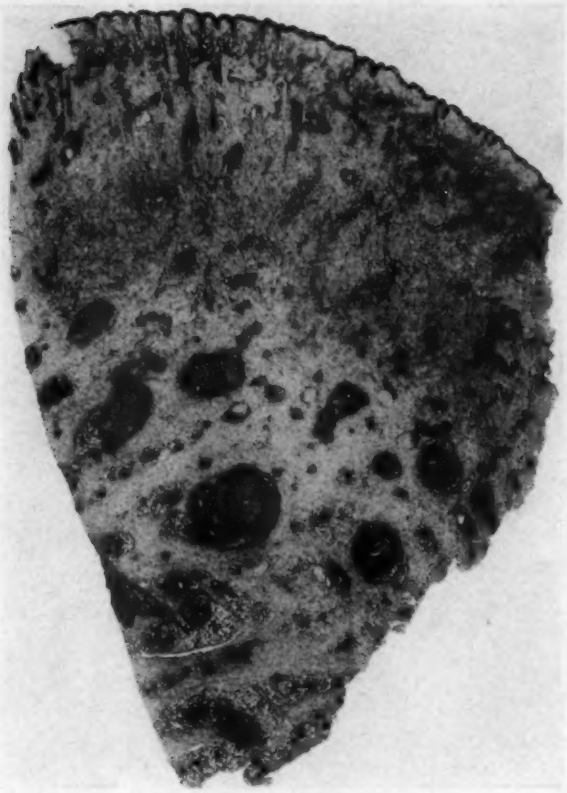

Fig. 3.—Extent of the local reaction following the intracutaneous injection of tuberculin into a naturally sensitized animal; × 6. The reactive process was severe and extended from the epidermis into the tissues of the subcutis.

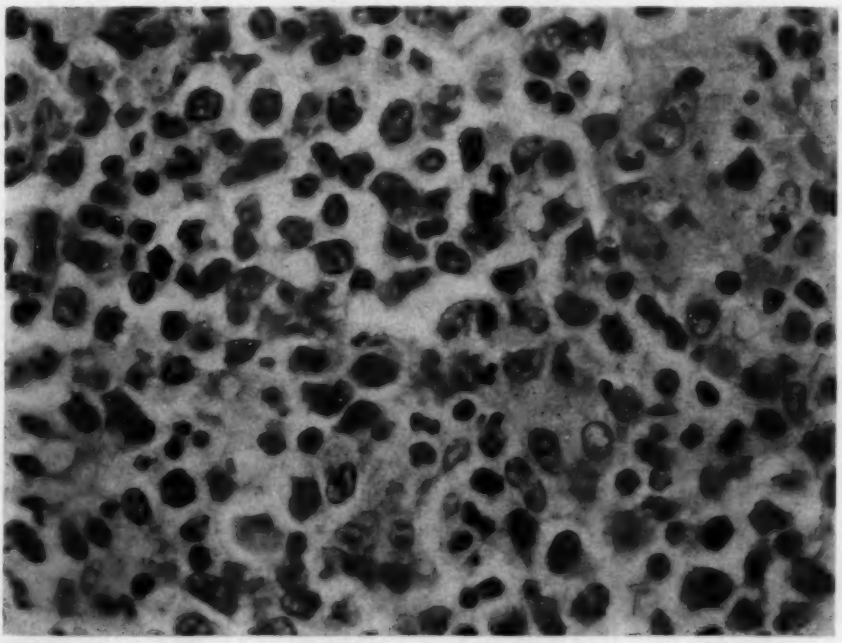

Fig. 4.—Diffuse collections of histiocytic cells, many of which are immature; × 660.

GROUP 2.—Five of the sixty-six specimens obtained from the caudal fold of the cattle which belonged to "the clinical group" and which had received a diagnostic intradermal injection of tuberculin were considered to be essentially normal when examined histologically.[15] The intensity of the reaction observed in the other sixty-one specimens varied greatly but revealed certain features that were common to all. In only a few of the specimens was the reactive process comparable in severity to that which occurred after the injection of mammalian tuberculin into calves that had been infected experimentally with bovine tubercle bacilli. Contrasting the reactions observed in "the clinical group" with those which occurred in "the experimental group," the reactions were considered slight in ten members of "the clinical group," of moderate degree in twenty-nine and well marked in twenty-two.

Histologically, the most constant feature of the reactive process was the perivascular and perineural occurrence of an excessive number of histiocytes, which had a tendency in many of the lesions to spread diffusely between the connective tissue bundles of the derma and to embrace both the papillary and the reticular zone of this structure (figs. 5 and 6). In severe reactions it was not uncommon for the reactive process to extend into the subcutaneous zone, and in four instances the reaction had extended beyond the subcutaneous tissues of the derma and involved the underlying muscle. The lesions of the musculature consisted of a diffuse infiltration of histiocytes between the muscle fibers and an edematous condition of the associated connective tissue.

The histiocytes were closely packed together, and the presence of mitotic figures indicated the proliferative activity of these cells. By appropriate stains[13] a delicate fibrillar reticulum was demonstrated the meshwork of which supplied a supporting stroma for the cells of the reactive process (fig. 7). A few lymphocytes could usually be recognized among the more numerous histiocytic cells, but polymorphonuclear leukocytes were not observed except in a limited number of specimens in which a single, small focal abscess had developed. Acidophilic granulocytes were demonstrated in fifty-one of the lesions. The majority of these cells were myelocytic, and true eosinophils were relatively few (fig. 8*A*).

Giant cells of the Langhans type were seen not infrequently among the histiocytic elements of the reaction (fig. 8*B*). In one instance a cell of this character was situated among the proliferating cells of the intima of a blood vessel. While these cells were never numerous, it was possible in a few instances to observe from three to five giant cells within the area of a single microscopic field.

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15. No lesions of tuberculosis were observed in any of these five animals at necropsy.

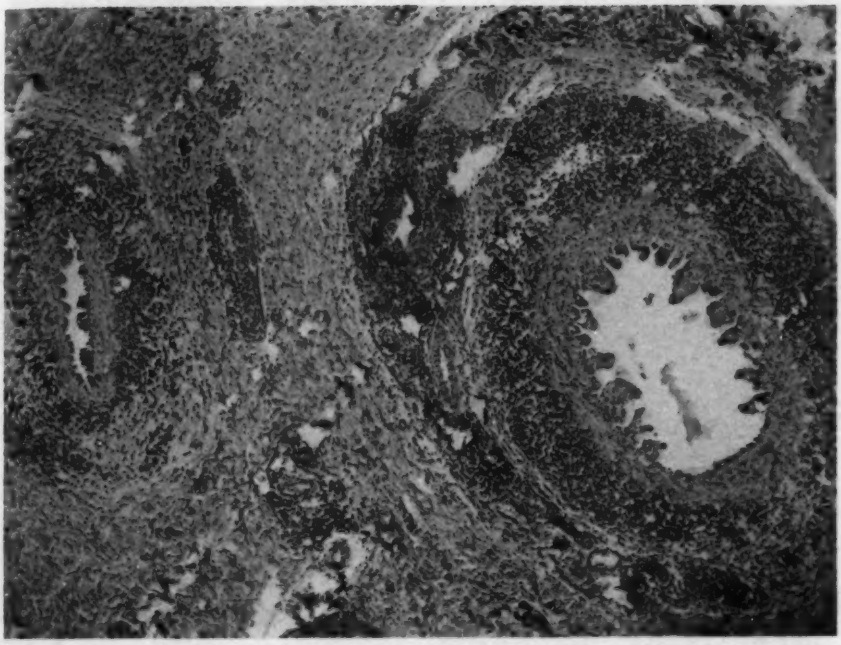

Fig. 5.—Perivascular proliferation of histiocytes, edema and endarteritis after the intracutaneous injection of tuberculin in a naturally sensitized animal; × 50.

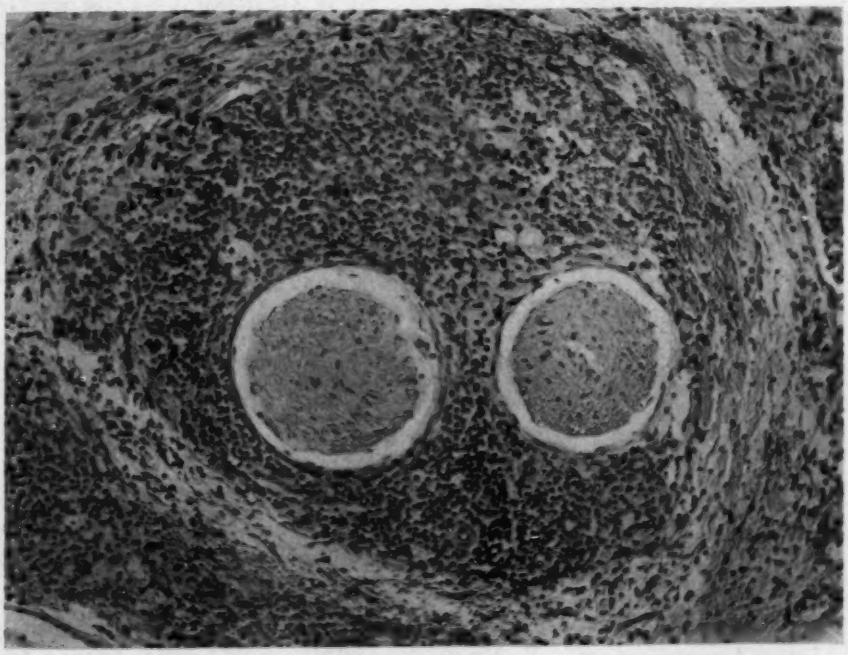

Fig. 6.—Perineural proliferation of histiocytes and lymphocytes after the intracutaneous injection of tuberculin; × 120.

Edema of varying degrees of intensity was present in thirty-four of the sixty-one reactions. The exudation was more noticeable in the reticular zone of the derma and in the subcutaneous tissues than it was in the papillary zone. In a few instances subendothelial edema was seen in the blood vessels which had brought about a separation of the endothelium from the media. Some reactions were observed in which edematous infiltration of the perineural sheath had occurred with a resultant bathing of the nerve cells. In the edematous portions of such nerves variable numbers of histiocytes were not infrequently seen. There

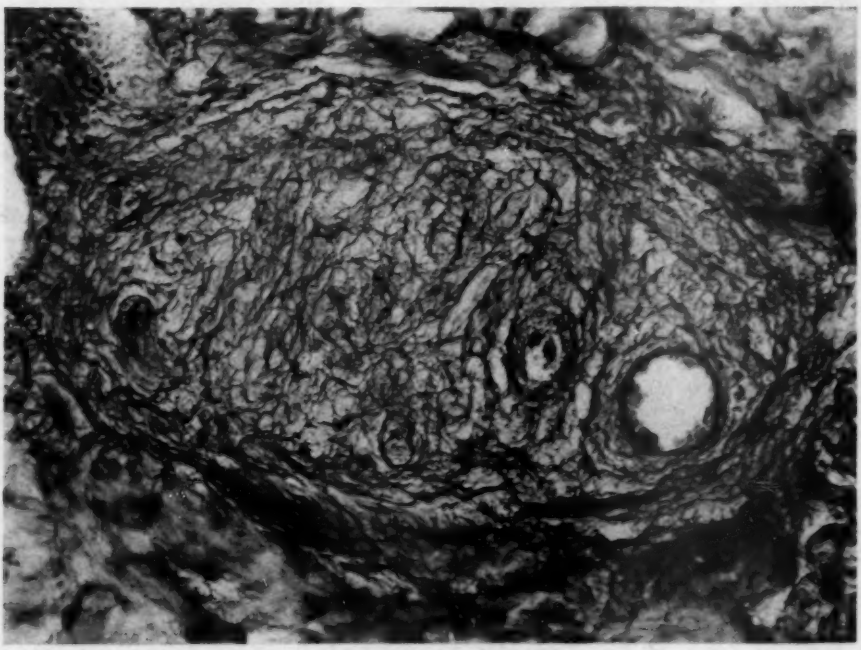

Fig. 7.—Delicate fibrillar reticular meshwork in an area of perivascular cellular proliferation after the injection of tuberculin; × 150.

was noted in a few nerves a moderate infiltration of histiocytes, but edema was absent.

One of the most interesting features of the histopathologic change was the ocurrence of various degrees of endarteritis of the vascular channels of the dermis. This was observed in twenty-eight of the reactions. This condition, when present, obtained usually in only one or two of the vessels, and the vessels in the deeper portions of the dermis appeared to be more frequently affected than those in the upper or papillary zone. The reactions, which differed widely in appearance, varied from a vigorously proliferative, canalized obstruction to a marked

thickening of the intima, interrupted by deep narrow clefts (figs. 9 and 10). The predominant cell entering into the reactive process was usually characteristically histiocytic in appearance, although endovascular lesions occurred in which the cells appeared cuboidal; in other lesions the cells resembled fibroblasts. Although none of the lesions of progressive endarteritis had induced complete obliteration of the lumen,

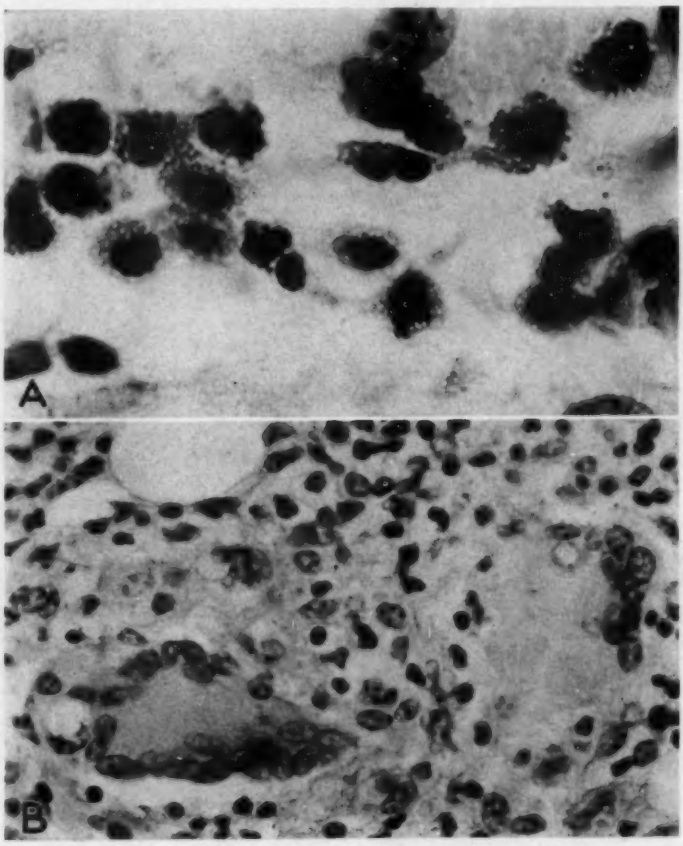

Fig. 8.—*A*, large number of acidophilic granulocytes in an area of tuberculin reaction; × 1,170. *B*, giant cells of the Langhans type in the midst of an area of cellular reaction; × 525.

thrombi were seen in fourteen of the specimens. When present, these vascular obstructions usually affected only a few of the vessels, although thrombi were numerous in some instances in which the reactive process was particularly severe. Organization was not observed in any of the thrombi. The character of the thrombosed vessels could not always be distinguished with certainty, but the majority appeared to be veins.

The endovascular changes in many instances involved only the intima, without morphologic alterations of the media. Many vessels, however, were observed in which the entire wall of the artery was affected. This occurred more often in those animals in which the reactive process was well marked or severe. Such an artery showed a conspicuous alteration of the muscular layer, which was attributable to the presence of small or large packets of histiocytic cells. These had penetrated the media and apparently had had their inception in the cellular hyperplasia of the perivascular zone (fig. 9). In some arteries the entire muscular

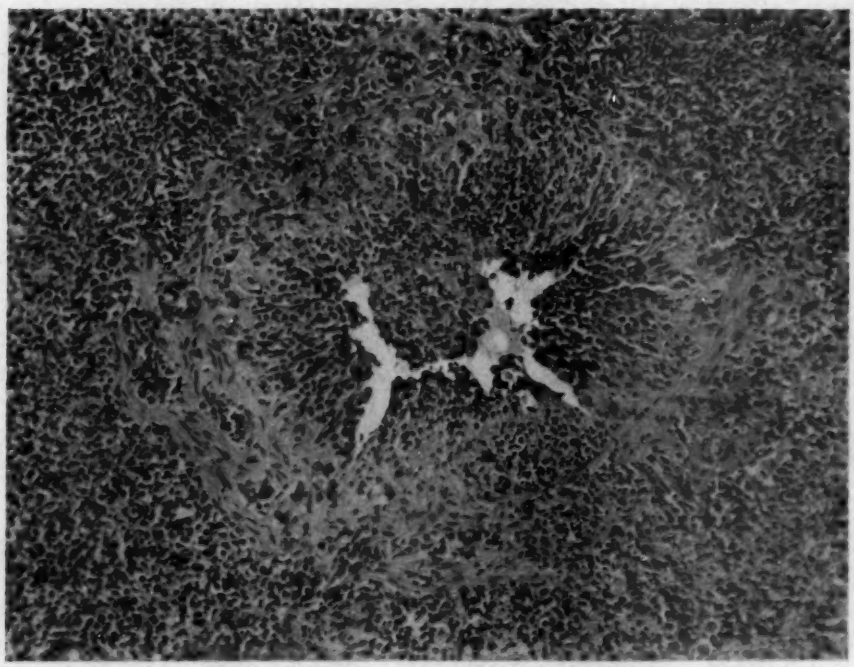

Fig. 9.—Marked endarteritis with infiltration of the vascular wall by the cells from the perivascular zone of proliferation; × 110.

layer was permeated with histiocytes, and it appeared that what was primarily a periarteritis became by continuity of the process an endarteritis. The excessive accumulations of histiocytic cells which had their origin in the perivascular tissues of the smaller blood vessels not infrequently nearly obliterated the lumen, and in some instances foci of histiocytic cells occurred in which no vascular structure could be recognized.

Cross-sections of six of the specimens of the smaller vessels showed an intravascular excess of leukocytes, although it is problematic whether

there existed a generalized leukocytosis, since this condition did not obtain in the larger vessels. Congestion of the blood vascular bed was not a conspicuous feature of the reactive process, although small areas occurred in which there were many extravascular erythrocytes suggestive of hemorrhage.

Necrosis of the epidermis was observed in few of the specimens, while retrograde changes were noted in different portions of the derma

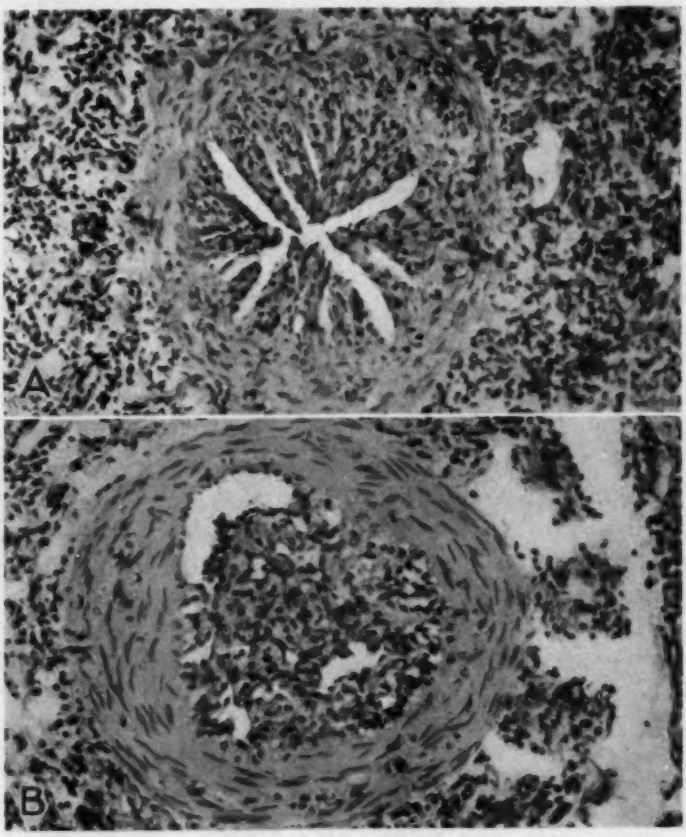

Fig. 10.—*A*, proliferative endarteritis of an artery in the derma after the injection of tuberculin; × 150. *B*, endarteritis of a vessel in the derma and marked thickening of the intimal layer; × 140.

in seventeen of the sixty-one lesions examined. These changes, which were usually limited to small areas, were characterized by pyknosis, fragmentation of the nuclei and other features indicative of early necrobiosis.

COMMENT

Two facts appear pertinent from the data obtained in this study: First, the relative intensity of a so-called positive local reaction following the intradermal injection of a diagnostic dose of tuberculin is no criterion as to the extent or severity of the tuberculous process in the sensitized animal. The tuberculin reaction in cattle with extensive lesions of tuberculosis was frequently mild or moderate, and comparable or even more severe reactions occurred frequently among the animals in which lesions of tuberculosis were not seen. Second, the histologic picture of the local reactive processes following the administration of tuberculin showed essentially the same pathologic changes regardless of whether or not lesions of tuberculosis could be seen at necropsy. Variations in the intensity or severity of the inflammatory process were frequently encountered, but the elements entering into the cellular reaction and the resultant pathologic pattern were remarkably consistent. There always occurred multiple proliferative foci of histiocytes which exhibited a characteristic predilection for the adventitial zones of the blood vessels and the perineural tissues. The histiocytic cells predominated in all the reactive processes, and while lymphocytes were usually demonstrable in variable numbers, polymorphonuclear leukocytes were significantly absent except in a few instances in which small abscesses had developed. Giant cells of the Langhans type were usually present, as were acidophilic granulocytes. Alterations of the vessels of the blood vascular system occurred sufficiently often to indicate that such changes are frequently part of the reactive process which ensues in the dermis when sensitized tissues are brought in contact with tuberculin.

Although edema was a prominent feature of many of the local reactions, this phenomenon was absent in twenty-seven of the sixty-one specimens in which the cellular constituents of the reactive process were present. It is recognized, of course, that had the clinical group been examined earlier edema might have been observed in a larger number of lesions, for undoubtedly edema occurred to some extent in the early phase of the reaction in all instances in which the intradermic test was recorded as positive. However, we are impressed with the fact that the local swelling in the focal or circumscribed type of reaction is attributable in no small measure to hyperplasia of the cellular elements, the histiocytes in particular, and is not necessarily dependent on the presence of a serous exudation.

While it is frequently fallacious to make analogous comparisons between clinical and experimental material, the histopathologic picture observed in the clinical material in this study seems to resemble closely that described by Laporte and by Dienes and Mallory, who studied the intradermic tuberculin reaction in guinea-pigs. Our observations are

incomplete in that they were limited to reactions in the later phase of the inflammatory response, whereas the investigators whom we have just mentioned followed the reaction from its inception to its maximal phase.[16] It is of particular interest to note that Laporte observed vascular alterations which were comparable in character to those which occurred in the material we studied. In our material the histogenesis of the cells constituting the proliferative type of endarteritis was not always obvious. In some instances it was clearly evident that the histiocytes in the perivascular zone had penetrated the muscular wall of the vessel and in this way had contributed to or initiated the endovascular changes. In others, however, no sign of such continuity of the reactive process was observed although serial sections were studied. For these changes Laporte's observations suggest a possible explanation. He described in the early stage of the intradermic reaction in guinea-pigs changes in the vascular endothelium in which the endothelial cells became swollen and showed a tendency to project into the lumen and to undergo mitotic division. While we failed to demonstrate mitosis in the endovascular cellular accumulations, these structures gave the impression of a proliferative process that had its origin in the elements of the intima.

SUMMARY AND CONCLUSIONS

In a histologic study of the later phases of the intradermic reaction to tuberculin in cattle experimental and clinical material was used. In seventy cases tissue was excised from the reactive areas of the caudal fold, and sections were prepared for histologic study. It was found (1) that the reaction to tuberculin is a rather definitely cellular reaction, predominantly histiocytic, and that it shows a predilection for the perivascular and perineural areas of the derma; (2) that while the local reactive process is confined in most instances to the papillary and reticular zones of the derma it may, if the inflammatory response is severe, extend into the tissues of the subcutis; (3) that there is no correlation between the intensity of the histologic alterations and the results of the tuberculin test as observed clinically; (4) that there are no distinguishing criteria in the histologic picture to indicate whether demonstrable lesions of tuberculosis are present in a given animal, and (5) that in all cattle in which these characteristic changes occur the uniformity of the reaction suggests the possibility of the existence of a common or closely related sensitizing agent.

16. An additional experiment is in progress in which early phases of the development of the intracutaneous tuberculin reaction in a group of calves are being studied.

EPIDEMIC ENCEPHALITIS IN JAPAN

THE CAUSATIVE AGENT COMPARED WITH THAT IN THE ST. LOUIS EPIDEMIC

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Epidemic encephalitis, a disease known in Japan as summer encephalitis (Futaki [1]) or encephalitis type B (Kaneko and Aoki [2]), appears with more or less regularity late in the summer or early in the fall and spreads in an explosive manner until an epidemic develops, which is characterized by a high mortality rate (over 50 per cent).

As for the causative agent, no reliable reports of its isolation are at hand, except that published by Hayashi,[3] in spite of strenuous efforts made by many research workers. Hayashi used for his inoculation experiments the brains of five patients who had died of typical encephalitis. He injected the emulsified and diluted brain tissue into the cranial cavities of monkeys (Macacus cyclopis). In five series of inoculations only one monkey showed pronounced symptoms of the disease, which could be observed also in subsequent passages through other monkeys of the same kind. The virus was passed through thirty-three animals for five passages, apparently without any decrease of virulence. The clinical as well as the pathologic picture in these diseased monkeys showed a close likeness to that of encephalitis in man. Unfortunately Hayashi did not carry the passages or his investigation beyond this point.

An epidemic of encephalitis broke out in Japan in 1935 with great severity, and from the beginning of August to the end of September fifty-two hundred cases had been reported throughout the country. Thus this epidemic rivaled that of 1923.

From the Pathologic Institute of the Niigata Academy of Medicine.

1. Futaki, K.: Nippon Densenbyo Gakkai Zasshi **3**:1207, 1929.
2. Kaneko, R., and Aoki, Y.: Ergebn. d. inn. Med. u. Kinderh. **34**:342, 1928.
3. Hayashi, M.: Proc. Imp. Acad. Japan **10**:41, 1934.

When the epidemic invaded the prefecture of Niigata, claiming a toll of about one hundred and eighty patients, we had the opportunity of making five autopsies and of using the brain tissue for inoculation experiments with a view to establishing some facts regarding the etiology of epidemic encephalitis in Japan.

INOCULATION EXPERIMENTS

The experimental animals used were young white mice weighing from 7 to 11 Gm.

The brain was removed aseptically at autopsy, and small pieces of tissue were excised from various parts (gyrus centralis, thalamus, aqueduct of Sylvius, substantia nigra, cornu ammonis, pons and medulla oblongata). The tissue was triturated in a sterile mortar and suspended in twenty times its volume of Ringer's solution. The animals were given intracranial injections of from 0.03 to 0.05 cc. according to their size and weight. The sterility of the material inoculated was checked by bacteriologic cultures in every case. Fresh material was used for

TABLE 1.—*Results of Inoculation Experiments*

Autopsy Number	Age of Patient, Years	Sex	Duration of Disease, Days	Time Between Death and Autopsy		Results of Inoculations
				Hours	Minutes	
1	15	F	8	21	25	Negative
2	4	F	5	26	30	Negative
3	5	F	6	..	45	Positive
4	50	M	8	1	20	Negative
5	60	M	5	2	25	Positive

inoculations, and small pieces thereof were placed in a 50 per cent mixture of glycerin and Ringer's solution and kept in an icebox for future use.

In each series from six to eight mice were used and were observed for three weeks.

From two (cases 3 and 5) of the five brains of patients who had died of encephalitis we were successful in isolating a virus and in maintaining its passage through animals. From the third patient a virus was obtained by inoculation of the material preserved in 50 per cent glycerin for nineteen days, while in case 5 inoculations both of fresh and of glycerinated material were successful. Material from the rest of the brains failed to produce the disease in mice when injected intracerebrally.

In case 5 autopsy disclosed only senile changes, i. e., arteriosclerosis with slight arteriosclerosis of the kidney, but in the brain and medulla there were changes characteristic of epidemic encephalitis. One mouse of each group was inoculated with material from the thalamus, from the gyrus centralis and from the pons and cornu ammonis, respectively.

Seven, seven and fourteen days, respectively, after inoculation the animals showed symptoms of the disease and died, thus showing that the virus was present in those parts of the central nervous system. Only one of the animals inoculated with the material preserved in glycerin became ill (on the twelfth day after inoculation with material from the cornu ammonis).

Only one of the animals inoculated with material from the third patient, died of the disease (on the thirteenth day after inoculation with glycerinated material originating from the pons). Inoculations with fresh material yielded no results in this case.

Further passages of the virus thus obtained were made by injecting material from the brains of the diseased mice into fresh animals, from the second passage on. The animals died within from four to six days after inoculation.

At present we are in possession of virus isolated from the gyrus centralis and the thalamus that has undergone from thirty to thirty-three passages, of glycerinated virus of material from the fifth patient that has undergone twenty-eight passages and of glycerinated material from the third patient that has undergone twenty-seven passages.

The disease in mice was manifested clinically in two forms. In some cases the animal became irritable at first and then exhibited tremor and jerky movements of the body. Later it sat in a corner of the cage, the hair bristled and the eyes half closed, and remained in a lethargic state (the lethargic form, fig. 1 *A*). The other, the paralytic form (fig. 1 *B*), was characterized by isolated or general paralysis of the extremities. A combination of the two forms also was seen in some of the animals, but they all died a few days after the onset of the disease.

At autopsy the animals showed more or less pronounced hyperemia of the cerebral meninges. Histologic examination revealed cellular infiltration of the leptomeninges in the brain and spinal cord, infiltration around the blood vessels and in the tissue, particularly in the gray matter (fig. 1 *C*), and reactive mobilization of the interstitial microglia cells (fig. 1 *D*). The cellular infiltration consisted predominantly of lymphoid cells, with which a few leukocytes with lobulated nuclei or large monocytes were mixed. Plasma cells also were often present in small numbers. Besides these changes, small, indistinct hemorrhages in the brain and spinal cord also were present. The pathologic changes noted in the central nervous system of the inoculated mice were typical of those of nonsuppurative meningo-encephalomyelitis.

If these observations are compared with those made in cases of epidemic encephalitis in man, it is seen at once that the meningitis, the inflammatory process of the blood vessels and brain tissue, the reactive changes of the glia (chiefly the microglia) cells and the degeneration of the ganglion cells throughout the entire brain and spinal cord are common to both. The pictures differ in that the accumulations of glia cells and neuronophagia, so common in human beings, were absent in the infected mice.

Experiments with material from both the third and the fifth patient proved that the virus passed through a Berkefeld N filter. The

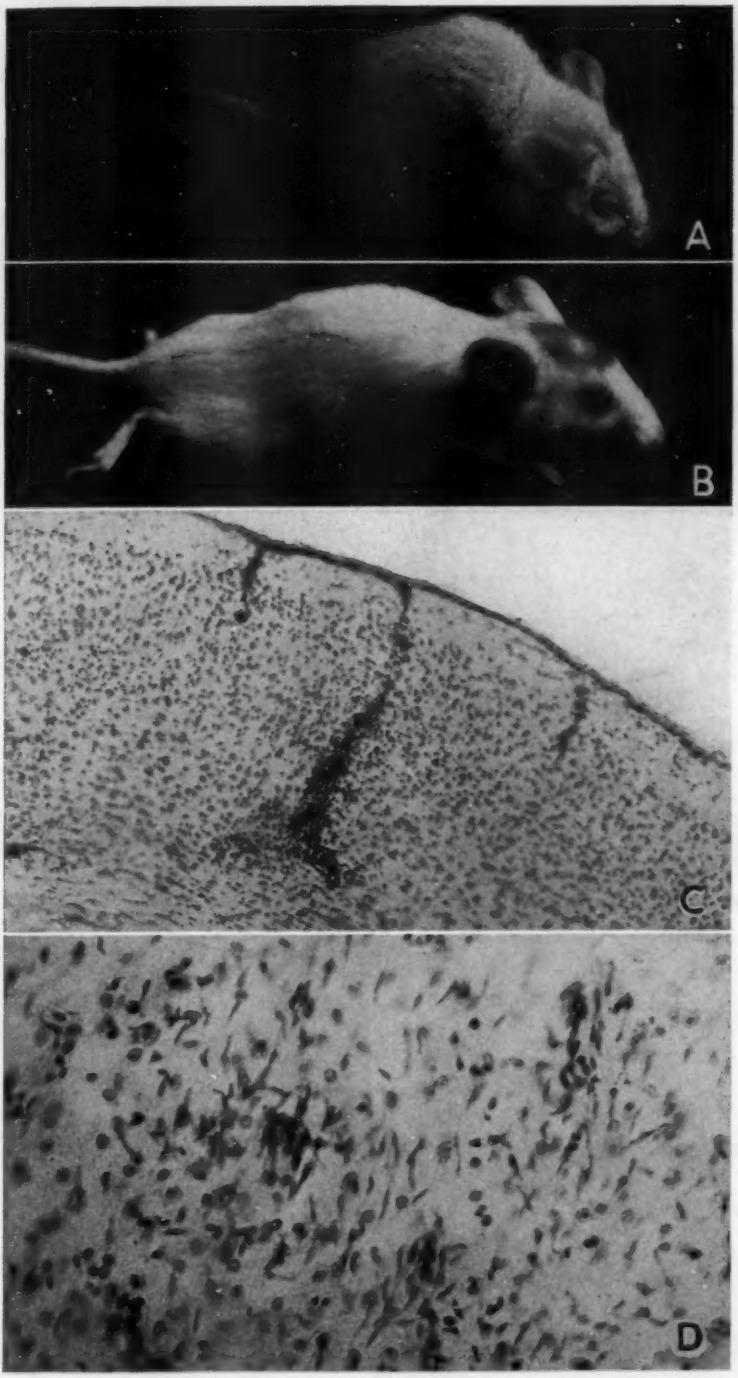

Fig. 1.—*A*, an animal with the lethargic form of encephalitis. *B*, an animal with the paralytic form of encephalitis. *C*, round cell infiltration of and around the blood vessels in the gray matter of the brain of a mouse with meningitis. Nissl's stain. *D*, rod cell proliferation in the brain stem (mouse). Nissl's stain.

animals inoculated with the filtrate invariably died, like the controls. The virus of Japanese epidemic encephalitis is therefore a filtrable virus.

In the following discussion the virus isolated from the fifth patient is referred to as Niigata strain 1 and that from the third patient as Niigata strain 2.

In order to test the infectivity of the isolated virus for animals other than mice, material from the brains of infected mice was emulsified in twenty times its volume of liquid and injected intracranially into dogs, cats, chickens, rats, rabbits, goats and monkeys. Besides the intracranial inoculation, subcutaneous, intraperitoneal and corneal inoculations by scarification were tried in rabbits. All these inoculations were controlled by simultaneous intracranial inoculation of mice. With the exception of mice and monkeys, none of the inoculated animals acquired the disease.

The condition as it developed in monkeys after intracranial inoculation with material from the brain of a diseased mouse was as follows:

Monkey 1, a male Macacus cyclopis, weighing 5,410 Gm., was given 2 cc. of virus strain 1 prepared in the usual manner.

Monkey 2, a female Macacus cyclopis, weighing 3,240 Gm., was given 1.5 cc. of virus strain 1.

Monkey 3, a male Macacus cyclopis, weighing 6,880 Gm., was given 2 cc. of virus strain 2.

After an incubation period of from six to seven days, the body temperature of all three monkeys began to rise, in a few days reaching 40.7 C. (105 F.). Each animal squatted in the darkest corner of its cage, with hair bristling. It took no food and appeared prostrated and somnolent, soon exhibiting convulsions. The first monkey remained under close observation for twenty-four days after inoculation and was then bled to death, so that all the blood could be obtained. The temperature reached its peak of 40.5 C. (105 F.) on the third day after inoculation and thereafter declined, reaching a normal level on the ninth day. The two other monkeys were killed at the acme of the fever or shortly afterward. Nothing of note was observed in the blood picture of these infected monkeys.

The brains of the monkeys killed at the acme of the disease showed slight hyperemia of the leptomeninges. Histologic examination revealed round cell infiltration in the meninges, in the walls of and around the blood vessels, both in the brain and in the spinal cord (fig. 2 *B*, *D* and *F*). These changes were located chiefly in the gray matter (polioencephalomyelitis). Diffuse reactive mobilization of the glia cells and nodular proliferation (fig. 2 *C* and *D*) also were seen but were not so pronounced. There were many neuronophagic figures (fig. 2 *D* and *E*), and in places they were distinct. Severe degeneration of the nerve cells was widespread.

The chief changes were (1) round cell infiltration of the meninges and tissues of the brain, cerebellum and spinal cord, (2) neuronophagia of the brain (mainly of the stem) and spinal cord and (3) degeneration

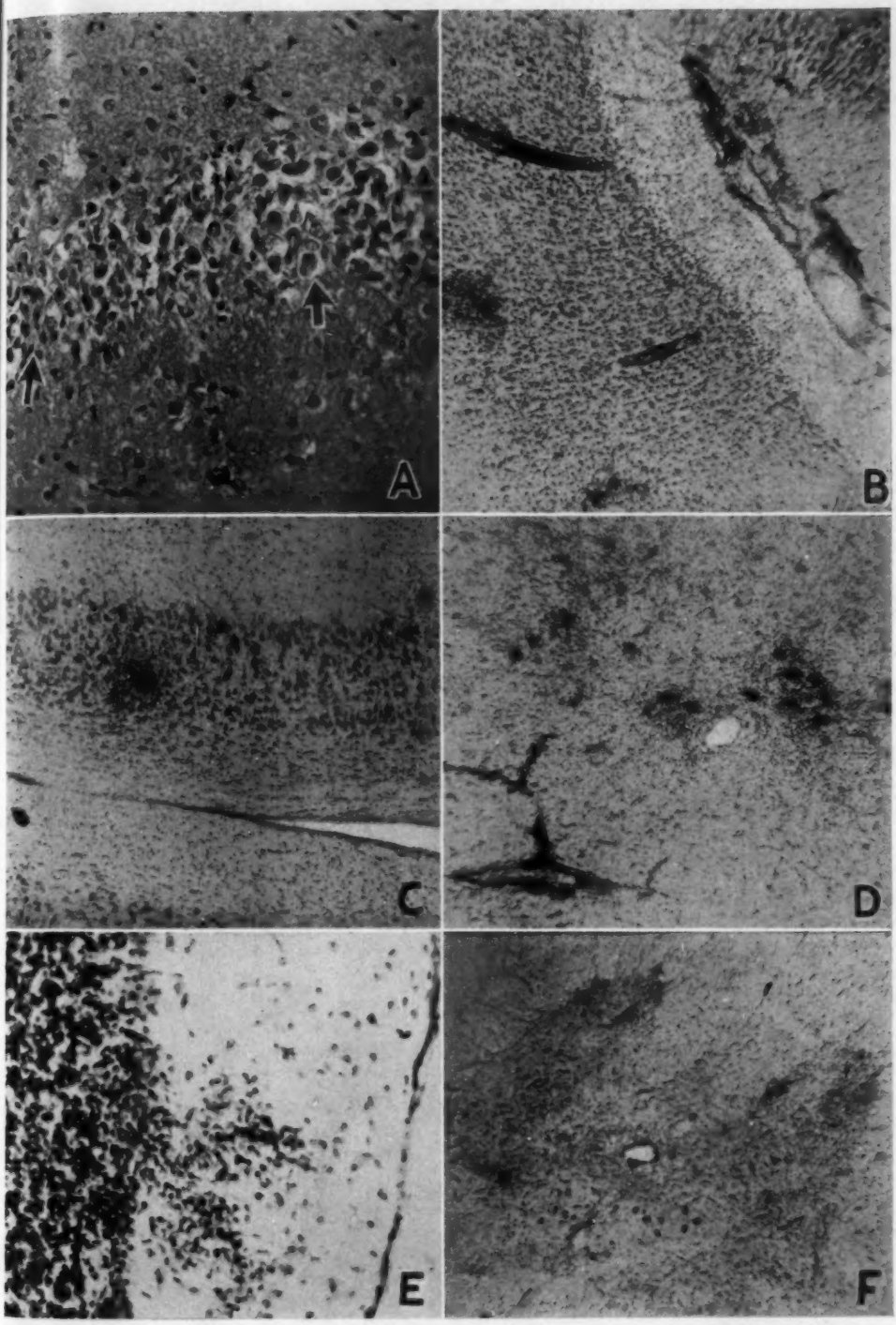

Fig. 2.—*A*, partial degeneration and necrosis (between the arrows) of the cell band of the cornu ammonis (mouse). Van Gieson's stain. *B*, round cell infiltration of the blood vessels and nodules of glia cells in the gray matter of the brain of monkey 2 with meningitis. Nissl's stain. *C*, nodules of glia cells in the cornu ammonis (monkey 3). Nissl's stain. *D*, perivascular round cell infiltration, neuronophagia with diffuse proliferation of glia cells surrounding it in the thalamus (monkey 2). Nissl's stain. *E*, so-called *Gliastrauchwerk* of Spielmeyer in the molecular zone of the cerebellum (monkey 2). Nissl's stain. *F*, neuronophagia, proliferation of glia cells in the anterior and lateral horns and perivascular infiltration in the white matter of the spinal cord (monkey 2). Nissl's stain.

of the ganglion cells of the brain and spinal cord and more or less diffuse, nodular proliferation of the glia cells, chiefly the microglia cells.

From these observations it is evident that the condition of the brains of the infected monkeys approximated that of the brains of human beings with meningo-encephalomyelitis much more closely than that of mice.

Monkey 1 (fig. 3) was bled at the height of the disease, and the blood was diluted one-half before being injected into mice. Monkey 2 was bled on the first day of the disease, and the blood was similarly diluted. On the fourth day, which was the first afebrile day, blood was taken again and diluted one-fifth. Monkey 3 was bled at the height of the infection, and the blood was diluted one-half. Each of these various samples of diluted blood was injected into ten mice intracranially. The results of all these inoculations were negative, in agreement with the experimental findings of Webster and Fite [4] with the St. Louis virus.

After monkey 2 (fig. 4) had been killed, samples of the gyrus centralis and the cornu ammonis were excised, emulsified, diluted 1:100 and injected into the brains of mice. Likewise specimens of various organs (salivary glands, liver, spleen

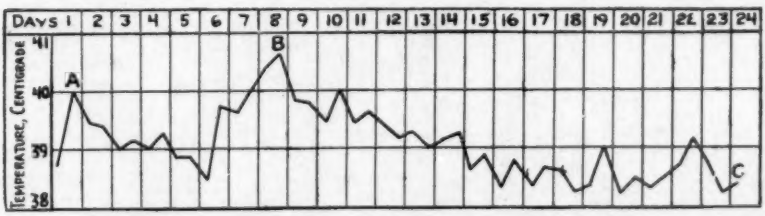

Fig. 3 (monkey 1 [Macacus cyclopis]).—At *A* the intracranial inoculation was given. At *B* there was edema of the eyelids. One cubic centimeter of blood was withdrawn. At *C* the animal was bled to death.

and kidney), diluted 1:10, were injected intracranially into mice. For the inoculations of each specimen of organ a series of ten mice was used. The animals that had been given the inoculations were observed for three weeks, but none of them showed any sign of sickness.

At autopsy on monkey 3, which had been inoculated with strain 2 intracranially, a brownish-red focus was seen in the brain at the point of inoculation. From this spot, from the gyrus centralis and from the thalamus, specimens were removed and diluted 1:20. Ten mice were then given intracranial inoculations of each material. One of the mice that was inoculated with material from the gyrus and one that was inoculated with material from the brownish spot showed symptoms of the disease in seven days.

Although the presence of the virus in the brains of monkeys has been established, it is at once evident that demonstration of the diseased condition is not as easily carried out in monkeys as it is in human beings at autopsy.

4. Webster, L. T., and Fite, G. L.: Science **78**:463, 1933.

In mice the condition seems to be different from that in monkeys, for we have succeeded in demonstrating the presence of the virus in mice (although not with absolute regularity) often not only in the brain but also in the blood, submaxillary glands, lungs, liver, spleen, adrenals and lymph glands. The virus that was proved to exist in the blood of mice is apparently not present, as a rule, in the blood of monkeys. Before this question can be decided, however, various factors must be considered, such as the species of the monkeys, the degree of infection of the donor of the virus and the amount of virus inoculated.

The infectivity, as established by the minimal infective dose, rose in the course of passages in mice. For instance, the minimal infectious dose in the fourth passage was 1 : 1,000,000, but that in the ninth pas-

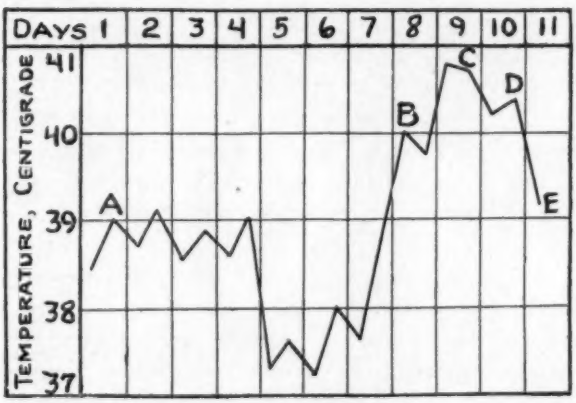

Fig. 4 (monkey 2 [Macacus cyclopis]).—At *A* the intracranial inoculation was given. At *B* 5 cc. of blood was removed. At *C* the animal had no appetite; it was prostrated and lethargic, with some tremor. At *D* the condition was a little improved, and the tremor was slightly reduced. At *E* the animal was killed, and samples from the organs were injected into the brains of mice.

sage was 1 : 10,000,000, and all the inoculated animals became infected by that time and died.

As it is known that the serum of a person convalescent from epidemic encephalitis neutralizes the virus, this postulate must be complied with before the virus isolated by us can be reasonably considered as the causative agent of the disease. The presence of a specific virucidal substance in the convalescent serum is a logical requirement.

In the beginning of our investigation we succeeded in demonstrating this phenomenon in two patients.

The patients were a woman aged 21, who at the time of the withdrawal of blood had been ill for twenty-eight days, and a 6 year old boy, from whom blood

was taken thirteen days after the onset of the disease. To the serums of these patients an equal part of Niigata virus strain 1 was added in the following dilutions of Ringer's solution: 1:1,000, 1:10,000 and 1:100,000. Six mice were inoculated in each series. The serum-virus mixtures, without the addition of an antiseptic, were allowed to remain in an incubator for two hours before the intracerebral inoculation was made. All the control animals, which received the virus and the serum of a healthy person, in dilutions of 1:1,000, 1:10,000 or 1:100,000, died from five to seven days after inoculation. In the first series three of those which received the 1:1,000 dilution of the virus and the convalescent serum, four which received the 1:10,000 dilution and all six which received the 1:100,000 dilution survived. In the second series one of the animals receiving the 1:1,000 dilution, two of the animals receiving the 1:10,000 dilution and four receiving the 1:100,000 dilution remained alive.

Encouraged by these results, we endeavored to obtain convalescent serum from persons in different parts of Japan, not only from those who had been ill during the recent epidemic but from those who had contracted encephalitis during previous epidemics. The results are recorded in table 2, which gives the data regarding the serums.

A total of eighteen patients were examined, ten males and eight females, whose ages ranged between 3 and 60 years. Although the neutralization power of the serums showed quantitative variations, there was not a single instance in which the convalescent serum did not neutralize the virus of epidemic encephalitis in one dilution or another. The following reactions were obtained: two strong (+++), eight medium strong (++), five weak (+) and one very weak (±). This proves that the serum of patients convalescent from epidemic encephalitis contains a virucidal substance, even though in some instances it may be weak. As the localities from which the serums originated were widely scattered throughout Japan, it is evident that the etiology of the disease in all the cases was the same. As the serum from the one patient who had encephalitis in 1931 showed a marked virucidal action against the virus isolated during the 1935 epidemic, it may be concluded that immune substances persist in the blood of convalescents for a considerable time. Since this patient had suffered from an unusually severe attack of encephalitis, it is possible that the titer of the serum depends on the severity of the disease, for no evidence can be gathered from our investigation that any relation exists between the strength of the serum and the age or the sex of the convalescent patient. The virulence of the virus in a particular epidemic and the susceptibility of the individual patient would naturally be expected to play a part.

The same virucidal power, although not so strong, could be achieved with the serum of monkey 1, which, as previously reported, received an intracranial inoculation of brain tissue of a diseased mouse, in spite of the negative result obtained in a control animal.

Therefore, as in other infectious diseases so in epidemic encephalitis, a certain duration of the disease is necessary before antibodies are present in the blood. As shown by our investigation, this time can be set at about three weeks. The following case illustrates this fact.

TABLE 2.—*Results of Neutralization Tests with the Niigata Strains and the Serums of Patients in Other Parts of Japan*

Virus Strain	Source of Serum	Epidemic	Patient Age, Years	Patient Sex	Days Between Onset and Bleeding	Results with Various Dilutions of Viruses* 10^{-4}	10^{-5}	10^{-6}	Result
Niigata 1	Tokyo	1935	7	M	28	9, 12, s, s, s, s	s, s, s, s, s, s	s, s, s, s, s, s	+++
	Yamanashi	1935	15	M	About 40	7, 9, 10, 10, 11, s	6, s, s, s, s, s	s, s, s, s, s, s	++
	Control	....	..	..	..	5, 6, 6, 7, 7, 7	6, 7, 8, s, p, p	7, 7, 8, 9, 11, 13	—
	Tokyo	1935	3½	M	24	6, 7, 7, 7, 8, 8	7, 7, 12, s, s, s	9, 10, s, s, s, s	+
	Okayama	1935	26	M	27	7, 7, 7, 8, 8, 9	6, s, s, s, s, s	s, s, s, s, s, s	++
	Okayama	1935	17	F	22	7, 7, 7, 7, 8, 8	6, 9, 9, 10, 11, s	s, s, s, s, s, s	+
	Niigata	1935	62	M	22	8, 8, 10, 12, s, s	10, 11, s, s, s, s	9, 12, s, s, s, s	+
	Niigata	1935	54	F	43	7, 7, 10, 10, s, s	7, 8, 10, 12, s, s	12, s, s, s, s, s	+
	Yamanashi	1935	15	M	28	8, 10, 12, s, s, p	7, s, s, s, s, p	s, s, s, s, s, s	++
	Yamanashi	1935	45	F	About 16	6, 6, 6, 6, 7, 7	7, 8, 9, 10, s, p	s, s, s, s, s, s	+
	Control	....	..	..	..	6, 6, 6, 7, 10, p	6, 6, 7, 8, 8, 11	7, 7, 8, 9, 10, s	—
	Fukuoka	1935	26	F	37	5, 6, 6, 7, 7, 7	7, s, s, s, s, s	.....	+ —
	Niigata	1935	66	F	About 40	7, 8, 8, s, s, s	s, s, s, s, s, s	.....	++
	Niigata	1935	21	M	26	6, 7, 7, s, s, s	s, s, s, s, s, s	.....	++
	Control	....	..	..	..	5, 6, 6, 6, 7, p	5, 6, 6, 6, 7, 7	.....	—
	Nagano	1935	7	F	26	7, 8, s, s, s, s	6, s, s, s, s, s	.....	++
	Control	....	..	..	..	5, 6, 6, 6, 7, 7	6, 7, 7, 7, 8, 8	.....	—
	Nagano	1935	17	M	58	6, 7, 11, s, s, s	10, 13, s, s, s, s	.....	++
	Nagano	1935	50	F	36	6, 10, s, s, s, s	7, s, s, s, s, s	.....	++
	Nagano	1931	54	M	About 5 years	12, s, s, s, s, s	s, s, s, s, s, s	.....	+++
	Control	....	..	..	..	6, 6, 7, 8, 8, 9	7, 7, 7, 8, 8, p	.....	—
Niigata 2	Niigata	1935	62	M	22	5, 6, 7, 9, s, s	6, s, s, s, s, s	.....	++
	Control	....	..	..	..	6, 6, 6, 7, 7, 8	6, 7, 7, 7, 8, p	.....	—

* The figures in the columns indicate the number of days of illness before death occurred; s indicates survival, and p, death in four days.

A 6 year old boy became severely ill with epidemic encephalitis. The blood serum obtained thirteen days after the onset of the disease was tested for neutralization with the Niigata virus strain 1. The following results were obtained: A 1 : 10,000 dilution killed all six mice in from six to nine days; a 1 : 100,000 dilu-

tion killed four in from six to eight days and the others survived; a 1:1,000,000 dilution killed three in six or seven days, the others surviving. A test of this patient's serum thirty-two days after the onset of the disease showed the following results: A 1:10,000 dilution of the virus killed four mice in seven days; a 1:100,000 and a 1:1,000,000 dilution each killed one mouse in nine days, all the others surviving. Used as a control, the serum of a healthy man showed no neutralization power, since all the mice inoculated with each dilution of the virus died in from five to nine days.

From the results of our investigation it appears justifiable to conclude that the cause of epidemic encephalitis in Japan is a virus which produces nonsuppurative meningo-encephalomyelitis which passes through a Berkefeld N filter and which is transmissible only to mice and monkeys. The virus obtained from these animals is neutralized by the serums of patients who have recovered from epidemic encephalitis.

Then the question naturally arises whether or not the virus of epidemic encephalitis in Japan differs from that of the St. Louis type.

COMPARATIVE STUDY OF JAPANESE AND ST. LOUIS TYPES OF EPIDEMIC ENCEPHALITIS

With respect to the clinical, epidemiologic and pathologic features, the Japanese and the St. Louis type of encephalitis represent a nosologic entity. Those who have studied the Japanese type agree on that point.

Inada,[5] Futaki,[6] Kaneko and Aoki and Kakinuma[7] studied the disease clinically. Ashizawa,[8] Hayashi,[9] Kaneko and Aoki, Kawakami,[10] R. Mori,[11] S. Mori,[12] Tabe,[13] Uchiyama[14] and Wake[15] studied the pathologic changes associated with this disease and Iimura[16] and Kaneko and Aoki its epidemiology in Japan, while the St. Louis epidemic was studied from the clinical and epidemiologic standpoint by Muckenfuss,[17]

5. Inada, R.: Jikken-Iho **8**:792, 920 and 1023, 1922.
6. Futaki, R., and Takagi, I.: Jikken Igaku Zasshi **13**:1171, 1929.
7. Kakinuma, K.: Encephalitis Epidemica, Tohodo, Tokyo, Aoyamás Nippon Naika Zensho, 1934.
8. Ashizawa, R.: Shinkeigaku Zasshi, vol. 22, no. 9, 1923.
9. Hayashi, M.: Allg. Ztschr. f. Psychiat., vol. 95, no. 1, 1931.
10. Kawakami, Z.: Tr. Jap. Path. Soc. **15**:112, 1926.
11. Mori, R.: Nippon Biseibutsugakkai Zasshi, vol. 20, no. 14, 1926.
12. Mori, S., and others: Nisshin Igaku Zasshi, supp., 1925.
13. Tabe, H., and others: Tr. Jap. Path. Soc. **20**:214, 1930.
14. Uchiyama, T.: Shinkeigaku Zasshi, vol. 35, no. 4, 1925.
15. Wake, I.: Nisshin Igaku Zasshi **23**:80, 183 and 354, 1933.
16. Iimura, Y.: Nisshin Igaku Zasshi **24**:1063 and 1206, 1935.
17. Muckenfuss, R. S.: Bull. New York Acad. Med. **10**:444, 1934.

Armstrong,[18] Bredeck,[19] Leake[20] and Neal[21] and its pathology by Muckenfuss and others.[22]

As far as the etiology is concerned, Muckenfuss, Armstrong and McCordock,[23] using monkeys, and Webster and Fite,[24] using mice, isolated a strain of filtrable virus from the St. Louis patients.

As Webster and Fite[25] failed to observe a neutralizing effect of convalescent serum from fifteen Japanese patients on the virus in cross-examination tests, it was thought possible that the Japanese strain of virus might be different from the American strain in that respect and that the differentiation of the two strains by immunologic methods might be possible.

As previously mentioned, we succeeded in isolating strains of the Japanese virus and, having obtained, through the courtesy of Dr. Webster, of the Rockefeller Institute, New York, the St. Louis virus strain (no. 3) and several convalescent serums, we proceeded to carry out comparative studies of the two strains.

It may be of significance to mention the fact, which we have established, that our strain of white mice is just as susceptible to the St. Louis strain of virus as to the Niigata strains. Young white mice were inoculated and became ill after an incubation of from four to six days, succumbing to the disease a few days after the onset.

As to the clinical and pathologic features, no particular difference between the two strains of virus was noted. In table 3 the results of our inquiry as to the smallest infective dose of either strain are given.

Our strain of mice showed in this experiment practically the same susceptibility toward the different strains of the virus.

Table 4 gives the results of our experiments in which the distribution of each virus strain in the blood and in organs other than the brain was investigated. This table shows clearly that in both instances the presence of the virus could be demonstrated not only in the brains of the infected mice but also in the blood (viremia) and in various organs. It is further evident from these experiments that the viremia which undoubtedly exists in the infected mice is not of high degree, as failures were noted following inoculation of material from various organs and

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18. Armstrong, C.: Pub. Health Rep. **49**:959, 1934.
19. Bredeck, J. F.: Am. J. Pub. Health **23**:1135, 1933.
20. Leake, J. P.: Am. J. Pub. Health **23**:1140, 1933.
21. Neal, J. B.: Am. J. Pub. Health **23**:1144, 1933.
22. Muckenfuss, R. S.; Hempelmann, T. C.; McCordock, H. A., and Rivers, T. M.: Am. J. Pub. Health **23**:1148, 1933.
23. Muckenfuss, R. S.; Armstrong, G., and McCordock, H. A.: Pub. Health Rep. **48**:1341, 1933.
24. Webster, L. T., and Fite, G. L.: J. Exper. Med. **61**:103 and 411, 1935.
25. Webster, L. T., and Fite, G. L.: Science **79**:254, 1934.

of blood, although much higher concentrations were used than is necessary to prove the presence of the virus in the brain. Furthermore, the success of infection by inoculation of blood and material from various organs, with the exception of brain substance, is decidedly in favor of the St. Louis strain. However, no great importance can be seen in this difference, in our opinion. It is interesting that when both strains were used the virus seemed to be more concentrated in the adrenals than in the other organs.

As far as the portal of infection and the infectivity of the two strains are concerned, no information was obtained as all the tests were performed in the same way.

TABLE 3.—*Data on the Smallest Infective Doses*

Virus Strain	Results with Various Dilutions of Viruses*			
	10^{-3}	10^{-4}	10^{-5}	10^{-6}
St. Louis 3 (6 mice in each group)...	5, 5, 5, 6, 6, 7	5, 6, 6, 6, 7, 7	6, 6, 6, 8, 9, s	7, 9, s, s, s, s
Niigata 1 (4 mice in each group).....	4, 4, 5, 5	6, 6, 6, 7	6, 6, 7, 8	7, 7, 9, s

* The figures in the columns indicate the number of days of illness before death occurred, and s indicates survival.

TABLE 4.—*Data on the Virus Obtained from Different Organs*

Organs Tested	Dilutions of Virus	Days Between Inoculation and Death*	
		St. Louis Strain (2 Mice)	Niigata Strain (4 Mice)
Brain	1:1,000	6, 7	5, 5, 5, 6
Blood	1:2	6, 6	8, 8, s, p
Submaxillary gland	1:10	6, 6	5, 7, 7, p
Lung	1:10	6, 6	6, 8, s, p
Liver	1:10	5, 6	7, s, s, p
Spleen	1:10	6, 7	7, 10, s, s
Kidney	1:10	6, 7	8, s, s, p
Adrenal gland	1:10	5, 5	5, 5, 6, 6
Lymph gland	1:10	p, p	5, 7, 8, s
Testicle	1:10	5, 6	p, p, p, p

* s indicates survival; p, premature death.

No fundamental difference was found between the two strains of encephalitis virus. The incubation period was longer after nasal inoculation than is observed after intracranial inoculations, and in spite of the use of larger doses in subcutaneous inoculations the infectivity of the virus was lower than when other modes of inoculation were used.

From the results of these experiments it is evident, as far as clinicopathologic observations on the virulence, the invasion by the viruses of the blood and various organs and the infectivity of the viruses when administered by various modes of inoculation are concerned, that there is no difference between the American and the Japanese strain of virus of epidemic encephalitis. The only difference between them so far discovered is in their behavior toward convalescent serums in cross-

neutralization tests, by means of which the two strains can be differentiated. The results of the cross-examination tests are given in table 5.

TABLE 5.—*Summary of the Results of Cross-Examinations*

Virus	Donor of Serum: Locality	Epidemic	Age, Years	Sex	Time Since Sickness	Dilutions of Viruses*: 10^{-3}	10^{-4}	10^{-5}	10^{-6}	Result
St. Louis 3	Okayama	1932	19	M	About 4 years	4, 4, 5, 5, 5, 6	5, 5, 5, 6, 6, p	5, 5, 5, 6, 6, 7	.....	—
	Control	....	..	..	.....	4, 4, 4, 5, 5, 5	5, 5, 5, 5, 6, p	5, 5, 5, 5, 6, p	.....	—
	Okayama	1932	32	F	About 4 years	4, 5, 5, 5, 5, 5	5, 5, 5, 5, 5, 6	5, 5, 5, 6, 6, 6	.....	—
	Control	....	..	..	.....	4, 4, 5, 5, 6, 6	4, 5, 5, 6, 6, 6	5, 5, 6, 6, 6, 7	.....	—
	Okayama	1932	47	M	About 4 years	4, 4, 5, 5, 6, 6	4, 5, 5, 5, 5, 5	4, 4, 5, 5, 6, p	.....	—
	Control	....	..	..	.....	4, 4, 5, 5, 5, 6	4, 5, 5, 5, 5, 6	4, 5, 5, 6, 7, 7	.....	—
	Okayama	1935	14	M	11 days	.....	5, 5, 6, 6, 6, 7	6, 6, 7, 7, 8, 8	.....	±
	Control	....	..	..	.....	.....	5, 5, 6, 6, 7, 7	5, 6, 6, 7, 8, p	.....	—
	Okayama	1935	27	F	22 days	.....	.....	4, 4, 5, 5, 6, 6	4, 5, 6, 6, 8, 8	—
	Okayama	1935	26	M	27 days	.....	.....	4, 5, 5, 5, 6, 6	5, 5, 6, 8, 8, p	—
	Okayama	1935	12	M	26 days	.....	.....	4, 6, 6, 6, 7, p	6, 6, 6, 7, p, p	—
	Control	....	..	..	.....	.....	.....	5, 5, 6, 6, 6, 7	5, 5, 6, 6, 7, 7	—
			Patient Number							
St. Louis 3	St. Louis	1933	220		.....	.....	.....	8, 8, 8, 8, 8, 8	8, 8, 8, 8, 8, 8	+++
	St. Louis	1933	257		.....	.....	.....	8, 8, 8, 8, 8, 8	8, 8, 8, 8, 8, 8	+++
	Control	....	...		.....	.....	.....	6, 7, 7, 7, 8, 9	7, 7, 7, 8, 8, 9	—
	St. Louis	1933	222		.....	.....	7, 7, 8, 10, 11, p	7, 9, 8, 8, 8, 8	9, 8, 8, 8, 8, 8	++
	Control	....	...		.....	.....	5, 5, 5, 5, 6, 7	5, 5, 5, 6, 7, 7	5, 5, 7, 7, 7, 8	—
Niigata 1	St. Louis	....	220		.....	4, 5, 5, 5, 5, 7	5, 5, 6, 6, 7, 8	5, 6, 7, 8, 9, 9	.....	—
	St. Louis	....	222		.....	4, 4, 5, 5, 6, p	5, 6, 7, 7, p, p	7, 7, 7, 8, p, p	.....	—
	St. Louis	....	257		.....	5, 5, 5, 6, 6, 6	5, 6, 6, 7, 9, 9	7, 7, 7, 9, 9, 11	.....	—
	Control	....	...		.....	5, 6, 6, 6, 7, 7	5, 6, 6, 7, 7, p	7, 7, 7, 7, 9, p	.....	—

* The figures indicate the number of days of illness before death occurred; s indicates survival, and p, premature death.

SUMMARY

On the basis of the results of the experiments given in table 5, we feel justified in claiming that the American and the Japanese strain of encephalitis virus, although identical in every other respect, are different with regard to antigenic properties, as revealed by the neutralization test, and that the Japanese virus is a new virus.

HEMORRHAGIC EXTRAVASATIONS INTO THE LEAFLETS OF THE ATRIOVENTRICULAR VALVES

THEIR RELATIONSHIP TO PULMONARY EMBOLISM

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AND
ADOLPH R. BERGER, M.D.
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Hemorrhagic extravasation into noninflamed atrioventricular valvular leaflets has recently been observed at necropsy in four persons at Bellevue Hospital. In one, ecchymoses of leaflets of the mitral and tricuspid valves accompanied similar extravasations beneath the mural endocardium. In the other three, the strict limitation of the endocardial ecchymoses to the leaflets of the tricuspid valve and the coexistence of embolism of the pulmonary artery suggested a relationship between the hemorrhagic extravasations and the embolism.

There are few reported observations of ecchymoses in noninflamed valvular leaflets of the human heart. Luschka [1] saw ecchymoses in the valvular leaflets of two adults: In an 18 year old youth who committed suicide by hanging, a small blood mass was found in the free border of a leaflet of the tricuspid valve and in the anterior semilunar aortic cusp. In a subject in whom death was due to apoplexy an ecchymosis was noted in one of the aortic leaflets near the annulus.

In the extensive literature on subendocardial hemorrhage [2] it is generally asserted that the hemorrhage is limited to the septal wall of the left ventricle and the papillary muscles. Among the protocols of 102

From the Department of Pathology of Bellevue Hospital and the Department of Pathology of New York University College of Medicine.

1. Luschka, H.: Virchows Arch. f. path. Anat. **11**:144-149, 1857.

2. (*a*) Hofmann, E. V.: Wien. med. Presse **38**:1490-1491, 1890. (*b*) Marx: Off. Ber. d. preuss. Med.-Beamten-Ver, **2**:71-73, 1903. (*c*) Sury, K. V.: Vrtljschr. f. gerichtl. Med. **40**:23-51, 1910. (*d*) Aschoff, L.: Virchows Arch. f. path. Anat. **213**:176-181, 1913. (*e*) Zum Winkel, K.: Ueber die subendocardialen Blutungen im menschlichen Herzen, Inaug. Dissert., Marburg, J. D. Kuster, 1914. (*f*) Ribbert, H.: Centralbl. f. allg. Path. u. path. Anat. **27**:545-548, 1916. (*g*) Szubinski: Zentralbl. f. Herzkrankh. **8**:61-64, 1916. (*h*) Berblinger, W.: Centralbl. f. allg. Path. u. path. Anat. **28**:1-8, 1917. (*i*) Mönckeberg, J. G.: Ergebn. d. allg. Path. u. path. Anat. (pt. 2) **19**:561-563, 1921. (*j*) Schmauss, H., and Herxheimer, G.: Grundriss der pathologischen Anatomie, ed. 20, Munich, J. F. Bergmann, 1932, p. 444.

instances of subendocardial hemorrhage tabulated by Geringer [3] there is but one in which valvular ecchymosis is recorded. In this instance, hemorrhage into a mitral leaflet was observed in a young woman who died with syphilitic aortitis and coronary ostial stenosis.

The localization of subendocardial hemorrhages to the septal wall of the left ventricle and papillary muscles is similarly emphasized in the reports of experimental studies in animals.[4] In slaughtered cattle subendocardial hemorrhages occur very commonly, and Stoll [5] has described their appearance in the leaflets of the mitral and tricuspid valves. The latter investigator has reported their occurrence in the mitral leaflets of slowly exsanguinated rabbits, and Külbs [6] has seen such ecchymoses in the leaflets of the mitral and tricuspid valves of dogs following trauma to the chest. Ebbinghaus [7] and Fraenkel [8] have described ecchymoses of valvular leaflets in man in instances of indirect trauma to the heart accompanying fatal blows on the thoracic wall.

Geipel [9] recently described two cases in which hemorrhagic extravasation into the leaflets of the tricuspid valve accompanied pulmonary embolism. He believed that the hemorrhages were attributable to trauma to the leaflets by the embolus during its passage through the tricuspid orifice. He stated that similar hemorrhages may occur in the absence of pulmonary embolism and referred to one instance of this nature.

The blood cysts in the cardiac valves of new-born infants are not to be confused with the hemorrhagic extravasations to be described. The former have been well known since the descriptions of Elsässer [10] and Luschka.[1] They appear as well circumscribed endothelial-lined cysts (Levinson and Learner [11]). Even when encountered in adults their identity with the blood cysts of infancy has been easily recognizable by histologic study (Bundschuh,[12] Wegelin [13]).

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3. Geringer, J.: Beitr. z. gerichtl. Med. **8**:105-148, 1928.

4. Rothberger, C. J.: Klin. Wchnschr. **7**:1596-1600, 1928; Wien. klin. Wchnschr. **42**:442-443, 1929. Külbs, F., and Strauss, H.: Klin. Wchnschr. **12**:933, 1933. Sacks, A.: Ztschr. f. Kreislaufforsch. **26**:733-743, 1934. Sury.[2c]

5. Stoll: Vrtljschr. f. gerichtl. Med. **47**:53-67, 1914.

6. Külbs, F.: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **19**:678-702, 1909.

7. Ebbinghaus, H.: Deutsche Ztschr. f. Chir. **66**:176-203, 1903.

8. Fraenkel, E.: München. med. Wchnschr. **1**:704-705, 1905.

9. Geipel, P.: Virchows Arch. f. path. Anat. **282**:67-98, 1931.

10. Elsässer, quoted by Luschka.[1]

11. Levinson, S. A., and Learner, A.: Arch. Path. **14**:810-818, 1932.

12. Bundschuh, E.: Frankfurt. Ztschr. f. Path. **6**:65-69, 1911.

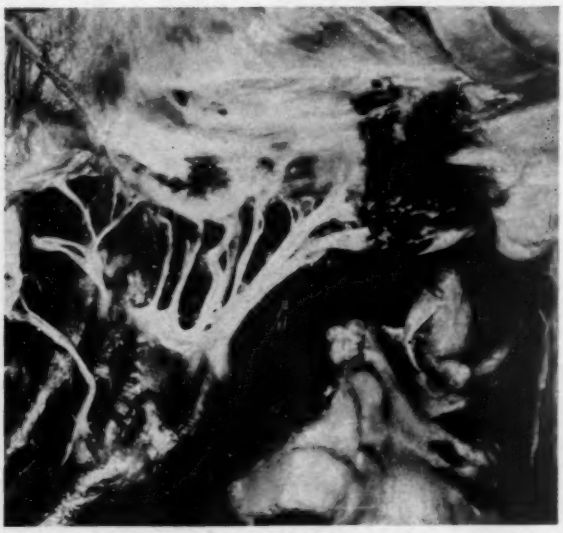
13. Wegelin, C.: Frankfurt. Ztschr. f. Path. **9**:97-141, 1912.

CASE REPORTS

CASE 1.—A 75 year old confused and disoriented white man was admitted to the psychiatric service complaining of trouble in his chest and presenting signs of congestive heart failure. Death occurred thirty-six hours later.

The anatomic diagnosis was: hypertrophy and dilatation of the heart, moderate coronary atherosclerosis, ecchymoses of the anterior leaflet of the tricuspid valve, pulmonary embolism and thrombosis, infected infarct of the left upper pulmonary lobe, arteriosclerosis of the brain and encephalomalacia, and arteriolar nephrosclerosis. The examination of the peripheral venous system was limited to the proximal 6 cm. of the femoral veins, and the latter were natural.

The anterior leaflet of the tricuspid valve presented an ecchymosis about 8 mm. in diameter, extending from the annulus to the free border (figure). Posterior to this were two smaller ecchymoses, one involving the annulus, the other situated about the midportion of the leaflet. The endocardium overlying the ecchymoses



Tricuspid valve, showing zones of hemorrhagic extravasation in the anterior leaflet (case 1).

was smooth. Serial microscopic sections through the affected leaflet revealed three discrete sites of hemorrhage, two involving the annulus and the third limited to the midportion of the leaflet. The extravasated red blood cells appeared well preserved, and there was a slight increase in the number of histiocytes and fibroblasts. The endothelium was intact, and there were no inflammatory changes. The blood vessels were strictly limited to the annulus and proximal 2 mm. of the cusp.

CASE 2.—A 76 year old confused white woman was admitted to the psychiatric service for dyspnea, hemoptysis and pain in the chest. Death occurred about twenty-four hours later, with signs of acute circulatory failure.

The anatomic diagnosis was: hypertrophy and dilatation of the heart, slight coronary atherosclerosis, ecchymoses of the anterior leaflet of the tricuspid valve, thrombosis of the right femoral vein, pulmonary embolism and thrombosis, and arteriolar nephrosclerosis.

Two discrete ecchymoses were visible in the anterior leaflet of the tricuspid valve. One, about 3 mm. in diameter, was confined to the distal half of the cusp; the other, about 5 mm. in diameter, was situated in its proximal half and less than a millimeter from the annulus. Serial sections revealed no continuity between the two hemorrhages, and blood vessels were encountered only at the annulus.

CASE 3.[14]—An elderly white man who was found dead in the street presented at necropsy femoral venous thrombosis, multiple pulmonary emboli and discrete hemorrhages 5 to 8 mm. in diameter in the distal portions of each of the leaflets of the tricuspid valve. Microscopic examination revealed extravasated red blood cells confined to the distal portions of the leaflets, and blood vessels were found only at the annulus.

CASE 4.—A 67 year old disoriented Negro was admitted to the psychiatric service in congestive heart failure and died several days later with severe respiratory distress.

The anatomic diagnosis was: marked hypertrophy and dilatation of the heart, ecchymoses of the leaflets of the tricuspid and mitral valves, subendocardial hemorrhages of the left auricle and ventricle, chronic passive congestion of the liver and congestion and edema of the lungs.

The leaflets of the tricuspid valve presented six discrete hemorrhages, varying from 1 to 10 mm. in diameter. One focus involved the annulus, but the others were confined to the middle or distal portions of the leaflets. There was an equal number of discrete smaller hemorrhages along the line of closure of the mitral leaflets. Blood vessels were demonstrated microscopically only at the annuli of the leaflets. There were large subendocardial hemorrhages in the left auricle, in the papillary muscles and in the septal and anterior walls of the left ventricle.

COMMENT

Hemorrhages into the leaflets of the tricuspid valve have been encountered in three persons who died of pulmonary embolism and thrombosis. In another subject, who died in congestive heart failure with hypertensive heart disease, multiple ecchymoses were found in the leaflets of the tricuspid and mitral valves and in the mural subendocardium.

What is the source of red blood cells which have been extravasated into the leaflets? Geipel [9] believed that in his cases rupture of the vessels in the annulus and proximal portion of the leaflet was followed by infiltration of the latter by red blood cells. The valvular hemorrhages experimentally produced by Külbs [6] were localized to the annulus and proximal portion of the leaflet, and in one specimen studied by serial sections rupture of a vessel was demonstrated. In a case of traumatic rupture of the heart reported by Ebbinghaus [7] one of the pulmonic leaflets exhibited an ecchymosis extending to the annulus, whereas in another leaflet the ecchymotic area was limited to the distal portion of the leaflet. Ebbinghaus thought that blood might be pressed into the

14. This case was brought to our attention through the courtesy of the Office of the Chief Medical Examiner of New York City.

leaflet following disruption of the continuity of the latter, but this was not morphologically demonstrated.

Though in all four cases some of the valvular ecchymoses involved the vascularized annulus and might have been due to rupture of those vessels, other hemorrhages were localized in the distal nonvascularized portions of the leaflets. The source of the red cells in the latter portions remains obscure. Though it is possible that cells from ruptured vessels at the annuli infiltrated the distal portions of the leaflets, no evidence of this was obtained even in serial microscopic sections. In view of the common persistence of vessels in the leaflets of the tricuspid and mitral valves (Wearn [15] and others) the possibility exists that the blood cells were derived from ruptured vessels which evaded our examination. Penetration by cells from the chamber of the heart through disruption of the lining of the leaflets is impossible to exclude, although we could not establish it by microscopic examination.

Are the ecchymoses of the leaflets of the tricuspid valve in cases 1, 2 and 3 related to the pulmonary embolism? That such ecchymoses may occur in the absence of embolism of the pulmonary artery is attested by the findings in case 4. However, the limitation of the hemorrhages to the leaflets of the tricuspid valve in the three cases of pulmonary embolism reported here, as well as in the two described by Geipel, supports the belief that the two phenomena are related. Whether this is due to trauma to the leaflet by the embolus during its passage through the tricuspid orifice or as the result of an abrupt rise in intraventricular tension cannot be determined.

Although the occurrence of subendocardial hemorrhages in the septal wall of the left ventricle and the papillary muscles is common and observed in many different conditions, their genesis is still obscure. The findings in case 4 are noteworthy only because valvular ecchymoses accompanied the mural subendocardial hemorrhages.

SUMMARY

Ecchymoses in noninflamed atrioventricular valvular leaflets were observed in four persons. In three who showed pulmonary embolism the ecchymoses were limited to leaflets of the tricuspid valve. In the fourth, hemorrhages occurred beneath the mural endocardium and in the leaflets of the mitral and tricuspid valves. In all four, some of the ecchymoses involved the vascularized annuli, whereas others were limited to the distal, apparently avascular, regions of the leaflets. It is suggested that the ecchymoses which were limited to the leaflets of the tricuspid valve were related to the coexisting pulmonary embolism.

15. Wearn, J. T.; Bromer, A. W., and Zschiesche, L. J.: Am. Heart J. **11:** 22-33, 1936.

EXPERIMENTAL PULMONARY EMBOLISM AND INFARCTION

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RELATION OF MULTIPLE PULMONARY EMBOLI TO DEATH

The presence of emboli in the smaller pulmonary arteries is not an infrequent discovery at carefully performed autopsies. Belt [1] found emboli within the pulmonary arterial tree and in the right chambers of the heart in 10 per cent of autopsies. The significance of emboli in these locations is questioned, but by many they are considered as a cause of death. McCartney [2] cited instances in which the occurrence of showers of small emboli or of a single large embolus was followed by death. Most of those who have made experimental investigations of the matter (Mann,[3] Dunn,[4] Underhill,[5] Hall and Ettinger [6]) have failed to induce death by pulmonary embolism unless practically the entire pulmonary circuit was closed.

The first part of this work deals with the introduction of emboli into the pulmonary arterial trees of dogs and the observation of the effects of the embolism on the behavior and periods of survival of the animals. The character of the emboli employed made it possible to watch their progress with the fluoroscope and roentgenograms. Pulmonary embolism was produced by the injection of lead shot (sizes 4 and 7½) and lead dust into the jugular vein. The number of shot varied between 5 and 350. Other substances such as blood clots, gummy and pliable resinous materials were tried, but no advantage was found in the use of them. Some of the animals were operated on under local anesthesia (procaine hydrochloride) to obviate any possible effects incident to general anesthesia. Other animals were anesthetized with pentobarbital sodium or ether. The operative procedure was carried out aseptically. As soon as the emboli were placed in the jugular vein their

From the Laboratories and the Department for Medical Research of Toledo Hospital.

1. Belt, T. H.: Am. J. Path. **10**:129, 1934.
2. McCartney, J. S.: Surg., Gynec. & Obst. **61**:369, 1935.
3. Mann, F. C.: J. Exper. Med. **26**:387, 1917.
4. Dunn, J. S.: Quart. J. Med. **13**:129, 1920; J. Physiol. **53**:v, 1919.
5. Underhill, S. W. F.: Brit. M. J. **2**:779, 1921.
6. Hall, G. E., and Ettinger, G. H.: Canad. M. A. J. **28**:357, 1933.

course was followed with the fluoroscope. The shot remained in the heart for periods of time varying from five minutes to the duration of the animal's life, embedded among the pectinate muscles. Most of the shot, however, passed into the pulmonary circulation within an hour. No particular predilection on the part of the emboli for either lung was observed, although a large number of the emboli gravitated to the middle and lower lobes. The animals were allowed to live for periods of from twenty-four hours to one and one-half years. No apparent ill effects from the presence of the emboli were observed except dyspnea on marked exertion in the animals with a larger number of shot. Our results, therefore, agree with those obtained by most of the other investigators. As far as the dog is concerned, under the conditions of these experiments multiple pulmonary emboli were found to produce neither death nor ill effects.

TABLE 1.—*Outcome Following Introduction of Pulmonary Emboli*

Dogs	Emboli Introduced into Arterial Pulmonary Circulation	Period of Observation	Effects of Embolism
5	25-350	1 to 1½ yrs.	No ill effects except for dyspnea on marked exertion in the animals with the greater number of emboli
10	75-150 and lead dust	6 mo.	No ill effects
20	75-150	3 mo.	No ill effects
15	75-150 and lead dust	1 mo.	No ill effects
25	75-250	1-3 weeks	No ill effects
30	75-250	1-7 days	No ill effects
5	5-10	1-14 days	No ill effects

EMBOLIC INTERFERENCE WITH THE PULMONARY ARTERIAL CIRCULATION

It is obvious that if infarction is to be produced all arterial supply to a given part must be shut off. To ascertain that such a result has been obtained, either the experimental procedure must have been determined previously to do this infallibly, or some follow up method must demonstrate that the desired end has been achieved. The mere introduction of foreign material into the pulmonary circulation and subsequent postmortem examination of the lung do not determine either the presence or the extent of circulatory interference unless an obvious infarct is found. In the absence of an infarct erroneous conclusions may be reached as to the relationship between emboli and infarcts.

Our method of demonstrating interference with the circulation consisted in introducing iodized oil into the jugular vein while the dog was alive. Within a minute after the injection the animal was killed. The lungs were removed, and a roentgenogram was made. This method utilizes the circulatory activity of the living animal and obviates possible artefacts which may be induced by injection methods applied to excised organs.

Iodized oil was injected into the jugular veins of normal dogs, and roentgenograms were taken of the excised lungs. The entire arterial tree including the finer branches was visualized. The animals in which lead shot emboli were introduced as outlined in table 1 were divided into two groups. In one group, consisting of 80 per cent of all the animals, iodized oil was injected into the pulmonary arterial circulation, and roentgenograms were taken. The following observations were made: Wherever emboli completely shut off the pulmonary arterial circulation there was lack of visualization of the arterial tree in that area. Both grossly and microscopically such an area was the seat of infarction. The size of the involved area, the location of the lesions

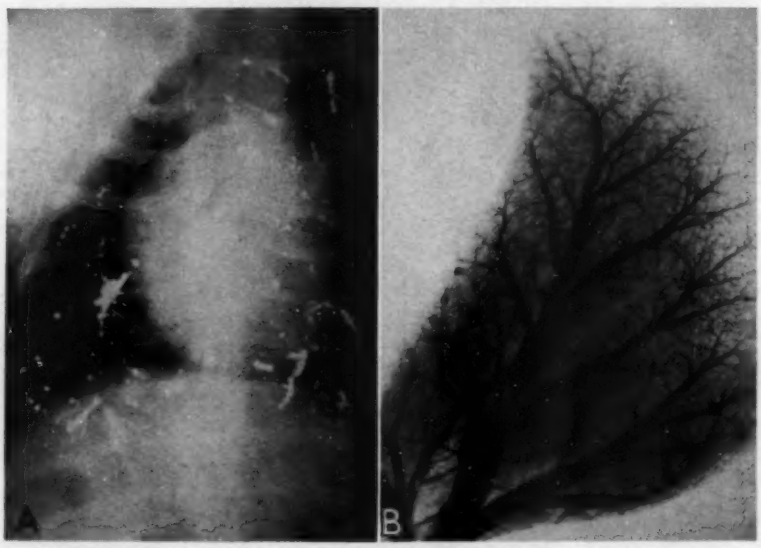

Fig. 1.—*A* is the roentgenogram of a dog showing the presence of lead shot emboli in the arterial pulmonary tree. *B* is the roentgenogram of a normal lung of a dog in which the pulmonary arterial tree received an injection of iodized poppy-seed oil while the animal was alive.

and the multiplicity of lesions in the lung played no part in producing infarcts. In areas in which there was only partial interference with the circulation the arterial visualization was scanty. Such areas apparently received a diminished supply of blood, the degree of visualization being proportional to the amount of blood entering the involved tissue. Correlation of these appearances with the gross and microscopic pictures of the lung revealed evidences of circulatory changes, which will be discussed later.

In other areas, although lead emboli occluded arteries, there was no interference with the blood supply. These areas, both grossly and

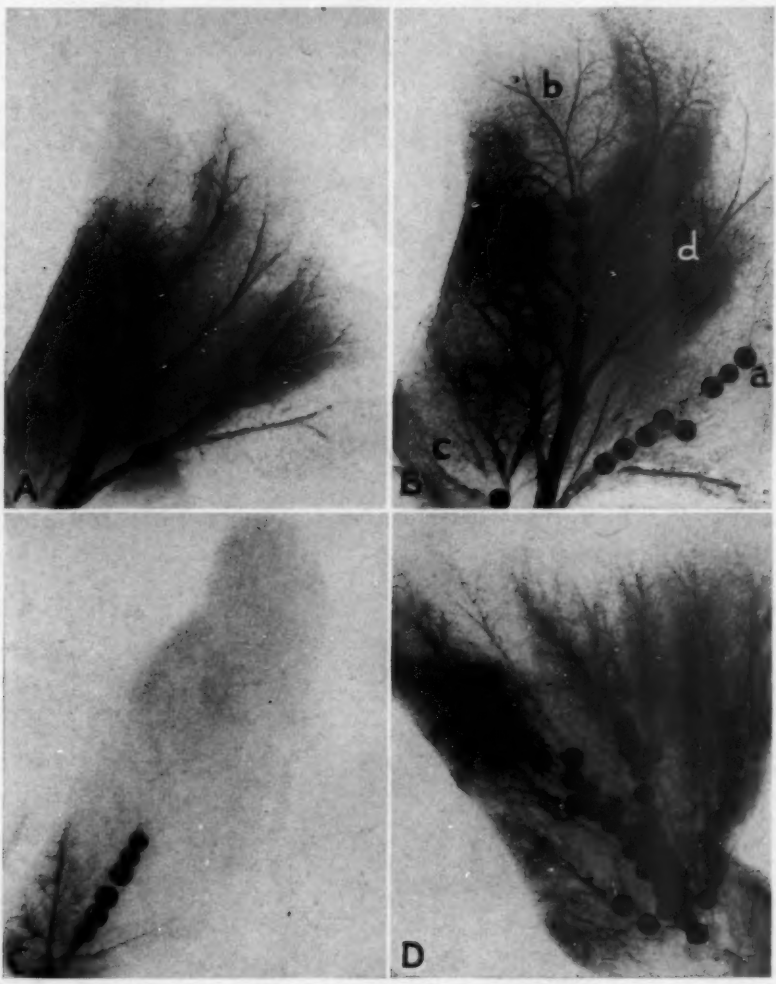

Fig. 2.—A pulmonary arterial tree which received an injection of iodized oil twenty-four hours (*A, B, C*) after the introduction of lead shot emboli. *A* shows a complete lack of blood supply in one area (gross infarct) and interference with most of the vessels in another area. *B* shows three varying degrees of interference with the blood supply in one lobe: (*a*) complete obstruction of the circulation and consequent lack of visualization with gross infarction; (*b*) and (*c*) two degrees of partial interference with the blood supply; (*d*) preservation of the blood supply despite the presence of an embolus. *C* shows practically an entire lobe without blood supply (grossly infarcted). *D* shows the pulmonary arterial circulation visualized with iodized oil four months after the introduction of lead shot emboli. There is visualization of the arterial tree peripheral to the emboli. The arborization, however, is scantier than in a normal lung. It is assumed that the circulation was restored in the area of lung previously devoid of all blood supply.

microscopically, showed no evidence of abnormalities. It was observed that rather a large number of emboli were necessary in a given part of the lung to produce any interference with the circulation. Frequently, as many as 50 shot failed to produce circulatory interference.

In from three weeks to a month after the introduction of emboli there was an apparent restitution of the pulmonary arterial circulation in the infarcted areas. Because of the limitations of our present method, such a restitution can be surmised only from the appearance of the emboli the presence of which had previously been observed to result in complete interference with the circulation. There is additional evidence that the pulmonary arterial circulation is gradually restored. While in the first four weeks after the introduction of pulmonary emboli 82 per cent of the animals showed complete closure of the blood supply, in the fifth week and thereafter the percentage gradually decreased until at the end of the year there was not a single instance.

Our findings explain the conclusions reached by some investigators who were unable to obtain infarcts with emboli alone. Karsner and Ash [7] introduced four turnip radish seeds into the pulmonary circulation and observed certain changes in the lung dissimilar to those characterizing an infarct. Their experiments led them to conclude that emboli alone do not produce infarcts. The changes found by these investigators were similar to those obtained by us in areas of the lung in which there was slight interference with the circulation. Apparently, the number of emboli introduced by Karsner and Ash was insufficient to close the pulmonary circulation completely to any given part.

Several investigators attempted to estimate the degree of obstruction of the pulmonary arterial circulation compatible with life. The earlier investigations of Lichtheim [8] and Welch,[9] substantiated later by the work of Mann,[3] Dunn [4] and others, indicated that a considerable number of emboli may be present without interfering with life. Haggart and Walker [10] found by compressing the pulmonary artery that closure of more than 52 to 66 per cent is necessary before circulatory failure and death ensue. Hall and Ettinger [6] occluded 75 per cent of the main pulmonary artery without causing death. The latter workers also failed to find in dogs any justification for the concept of an intrathoracic inhibitory reflex responsible for death as suggested by Karsner.[11]

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7. Karsner, H. T., and Ash, J. E.: J. M. Research **27**:205, 1912.

8. Lichtheim, L.: Die Störungen des Lungenkreislaufs und ihr Einfluss auf den Blutdruck, Breslau, F. W. Jungfer, 1876.

9. Welch, W. H.: Virchows Arch. f. path. Anat. **72**:375, 1878.

10. Haggart, G. E., and Walker, A. M.: Arch. Surg. **6**:764, 1923.

11. Karsner, H. T.: Human Pathology, Philadelphia, J. B. Lippincott Company, 1926.

By means of our method—the injection of iodized oil into the pulmonary tree of the living animal and the consequent visualization of intact and occluded pulmonary arteries—it was possible to estimate with some degree of exactitude the relation between the amount of lung tissue deprived of its blood supply and compatibility with life. The weight of the lungs was recorded. The lung tissue visualized with iodized oil and without such visualization was measured on the roentgenograms, and the total amount of tissue occluded was estimated. It was found that emboli might occlude enough of the pulmonary artery of a dog to deprive 79 per cent of the lung of its blood and the animal survive. Attempts to introduce more emboli for a greater degree of obstruction resulted in complete closure of the main pulmonary arteries and death.

From these experiments it is apparent that the mere introduction of foreign bodies into the pulmonary arterial tree does not presuppose complete closure of the blood supply to a given area of the lung. It is also obvious that it is necessary to introduce a large number of emboli before a part of the lung is deprived of the pulmonary blood supply. Consequently, failure to secure infarcts after injection of emboli may be due to the introduction of an insufficient number. The experiments further suggest that after three weeks the pulmonary circulation is gradually restored.

RÔLE OF THE BRONCHIAL CIRCULATION IN PULMONARY ARTERIAL CLOSURE

There are two problems concerning the relationship between the bronchial and the pulmonary arterial circulation: (1) the possible presence of communication between the two systems and the character of this if it exists; (2) the assumption by the bronchial arteries of the nutrition of the lung on closure of the pulmonic circulation. That an anastomosis exists between the bronchial and the pulmonary circulation has been maintained by many investigators (Virchow,[12] Henle,[13] Miller [14]). Miller [15] traced the bronchial vessels to the small bronchioles and believes that an anastomosis is established by a capillary network between the two systems. Karsner and Ghoreyeb [16] are of the opinion that the anastomosis takes place before the smaller divisions of the pul-

12. Virchow, R.: Gesammelte Abhandlungen zur wissenschaftlichen Medicin, Frankfurt, Meidinger Sohn & Co., 1856.

13. Henle, F. G. J.: Handbuch der systematischen Anatomie des Menschen, Braunschweig, F. Vieweg & Sohn, 1866.

14. Miller, W. S.: Anat. Anz. **28**:432, 1906.

15. Miller, W. S.: Am. Rev. Tuberc. **12**:87, 1925; Arch. f. Anat. u. Physiol. (Anat. Abt.), 1900, p. 197.

16. Karsner, H. T., and Ghoreyeb, A. A.: J. Exper. Med. **18**:500, 1913.

monary arteries are reached. Virchow [12] ligated the pulmonary artery and found the bronchial and the intercostal vessels enlarged, and because of the failure of the lung tissue to die he concluded that the bronchial arteries take over the burden of nourishment. Schlaepfer [17] substantiated Virchow's findings. Karsner and Ghoreyeb [16] stated that the bronchial arteries do not supply blood to lung tissue deprived of pulmonary circulation unless the bronchial blood pressure is extremely high, and that consequently only when embolism involves an entire lobe does the bronchial vessel play any part in nourishing the affected lung. These investigators further maintained that areas less than a lobe receive their blood supply by anastomosis of the pulmonary artery. Their statements are naturally predicated on their assumption that emboli alone do not produce infarcts.

To demonstrate the bronchial circulation we injected iodized oil into the left ventricular chamber of the heart of the living anesthetized dog, having previously tied both carotid and both subclavian arteries and having placed a ligature on the abdominal aorta. From one to two seconds after the oil was introduced the ligature was pulled up and the abdominal aorta tied. In normal dogs the bronchial tree was visualized only very faintly, but the arteries which give rise to the bronchial vascular system were outlined distinctly. Roentgenograms showed a complicated and variable origin from many of the intercostal and internal mammary arteries and from numerous small aortic branches. It was a convincing picture to the effect that any attempt to shut off the bronchial circulation by ligation is a technical impossibility.

A series of dogs (table 2) with lead shot emboli in the pulmonary arterial circulation were subjected to the injection of iodized oil into the bronchial arteries at varying intervals of from one day to six months after the production of the pulmonary embolism. The roentgenograms were compared with the gross and microscopic pictures. One day after the production of pulmonary embolism there was no change from the usual hardly perceptible bronchial visualization. On the second day the grossly infarcted areas showed on the roentgenograms faint visualization, which increased in intensity, reaching the maximum on the sixth day. The rest of the lung, grossly and microscopically normal, showed a hardly perceptible outline of the bronchial circulation. The size and the extent of the infarction played no part in the bronchial dilatation. Single small parts of one lobe or half the lung equally showed marked visualization of the bronchial arteries in infarcted areas. The lung tissue with partial obstruction of the pulmonary arteries showed only faint visualization.

17. Schlaepfer, K.: Am. Rev. Tuberc. **10**:35, 1924.

It is apparent that the bronchial arteries dilate wherever the pulmonary arteries are closed irrespective of the amount of lung tissue involved.

PREVIOUSLY RECORDED EXPERIMENTS ON EMBOLISM AS CAUSE OF PULMONARY INFARCTION

Cohnheim and Litten [18] were able to produce pulmonary infarcts by introducing emboli into the pulmonary arterial circulation. Fujinami,[19] Orth [20] and Zahn [21] confirmed Cohnheim and Litten's observations. Grawitz,[22] on the other hand, denied that any relationship exists between pulmonary embolism and infarction. Karsner and Ash [7] deduced from their experiments that an embolus without some other factor (passive

TABLE 2.—*Relation of the State of the Bronchial Circulation to Pulmonary Arterial Closure*

No. of Dogs	Time Between Introduction of Emboli and Visualization of Bronchial Tree with Iodized Oil	Appearance of Bronchial Tree After Injection of Iodized Oil
4	1 day	Visualization same as in normal lungs
4	2 days	Visualization faintly more pronounced in infarcted areas
4	4 days	Visualization fairly distinct in infarcted areas and same as normal elsewhere in lung
4	6 days	Visualization distinct in infarcted areas
4	2 weeks	Visualization well pronounced in infarcted areas and normal elsewhere in lung
3	3 weeks	Visualization well pronounced in infarcted areas and normal elsewhere in lung
3	4 weeks	Maximum visualization in infarcted areas and normal elsewhere in lung
3	6 weeks	Maximum visualization in infarcted areas and normal elsewhere in lung
3	3 months	Maximum visualization in infarcted areas and normal elsewhere in lung
3	6 months	Maximum visualization in infarcted areas and normal elsewhere in lung

congestion) is incapable of producing a true infarct if hemorrhage and necrosis are considered as changes characterizing an infarct. Most of the recent editions of textbooks on pathology accept the conclusions of the latter investigators.

PATHOLOGIC CHANGES IN LUNGS WITH MULTIPLE PULMONARY EMBOLI

Animals with pulmonary emboli (table 1 and 2) were killed at periods of from twenty-four hours to one and one-half years after the intro-

18. Cohnheim, J., and Litten, M.: Virchows Arch. f. path. Anat. **65**:99, 1875.
19. Fujinami, A.: Virchows Arch. f. path. Anat. **152**:61, 1898.
20. Orth, J.: Verhandl. d. Gesellsch. deutsch. Naturf. u. Aerzte **69**:7, 1897.
21. Zahn, F. W.: Verhandl. d. Gesellsch. deutsch. Naturf. u. Aerzte **69**:9, 1897.
22. Grawitz, P.: Ueber die haemorrhagischen Infarkte der Lungen, in Festschrift Rudolph Virchow zu seimem 71. Geburtstag gewidmet von den früheren und jetsigen Assistenten des Berliner pathologischen Instituts, Berlin, G. Reimer, 1891, p. 1.

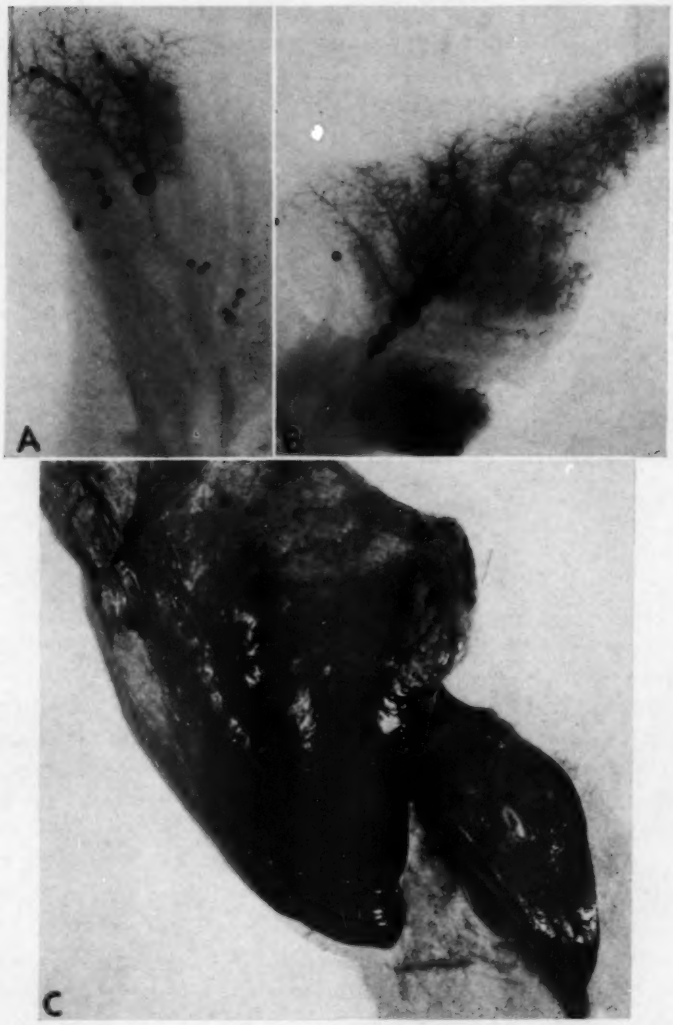

Fig. 3.—Iodized oil in the bronchial arteries two weeks after the introduction of pulmonary emboli. *A* shows one small part of one lobe of the entire lung involved. The area is grossly and microscopically infarcted. The visualization of the bronchial tree is assumed to be due to dilatation of the vessels. The iodized oil in the rest of the lobe is hardly perceptible, although the entire lung received the injection. *B* shows involvement of almost an entire lobe. Grossly the lobe is infarcted. Note the marked visualization of the bronchial tree and the lack of it in the noninfarcted base of the lobe. These photographs are believed to demonstrate that the bronchial vessels dilate in the presence of closed pulmonary arteries irrespective of the amount of lung tissue involved. *C* is the gross picture of an infarct of three days' duration. The affected area is black-red, raised and dry. The cut surface is deep red, firm and dry. Note the distinct demarcation between the infarct and the normal tissue.

duction of the lead shot. Diagrammatic drawings were made of the lungs to record: (1) the gross appearance, (2) the relative volume of normal and of abnormal tissue, (3) any correlation of the roentgenographic picture of the pulmonary and bronchial circulations with the gross and microscopic appearance of the lungs. Sections for microscopic study were removed from the abnormal portions of the lung, from the edges of normal and abnormal regions, and from presumptive normal lung tissue at some distance from the involved regions. The sections were stained with hematoxylin-eosin, Weigert's elastic and Brilmyer's [23] connective tissue stains.

Several types of lesions were observed. Correlation between the pathologic picture and the visualized pulmonary and bronchial circulations demonstrated that the type of abnormal change followed the degree of pulmonary arterial obstruction and bronchial vessel dilatation. Tissue completely devoid of pulmonary arterial blood and with dilated bronchial arteries presented grossly slightly elevated subpleural deep red tissue, triangular-shaped when located peripherally and irregular in outline when located within the parenchyma, with a black-red, dry, firm cut surface. This picture appeared within twenty-four hours after the introduction of the emboli and persisted in most animals for three weeks and in a few for three months. The microscopic changes within twenty-four hours consisted of the presence of massive amounts of blood within alveoli and in dilated capillaries and larger blood vessels. There was no destruction of either connective or elastic tissue. Within forty-eight hours and thereafter some of the red blood cells showed disintegration; the alveolar mucosa was denuded of many of its cells, and these showed nuclear degeneration. The elastic tissue was decreased, and there was some fragmentation of the connective tissue. In all instances, the bronchi and their blood vessels showed up as distinct intact islands in an area of extravasated blood. The bronchial vessels were uniformly dilated. This microscopic picture remained for periods of from three weeks to three months with a lesser or greater degree of the same character of abnormal changes. From three weeks on, the blood in the alveoli decreased, and eventually the lung tissue showed various degrees of fibrosis, largely interstitial and occasionally involving an area of alveoli.

Both grossly and microscopically the picture described of an area of the lung with a completely obstructed pulmonary arterial circulation is that of a hemorrhagic infarct. Karsner [11] gave an excellent and concise definition of an infarct as a "series of events following obstruction of a supplying vessel of a part, ultimately leading to necrosis." The gross and microscopic changes described represent "the series of events ultimately leading to necrosis." The absence of the eventual complete

23. Brilmyer, G. J.: Science **68**:114, 1928.

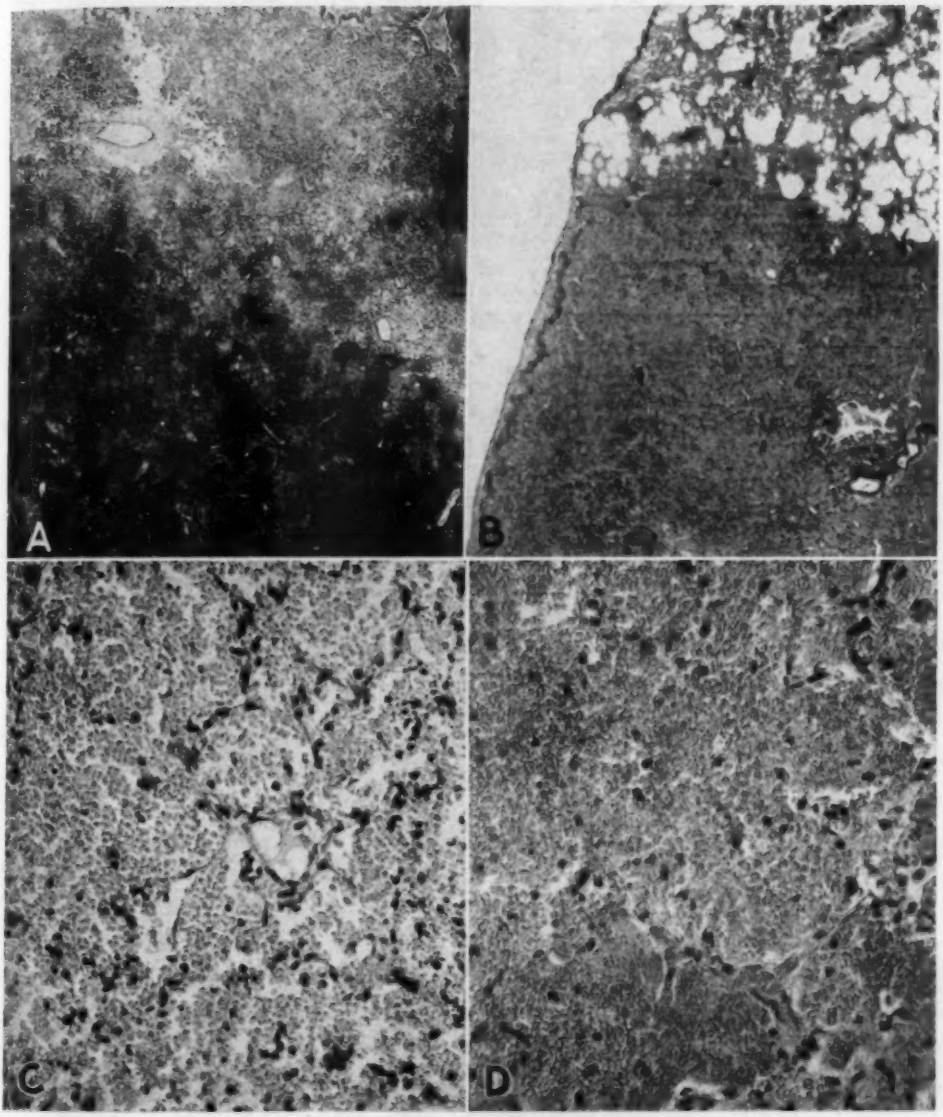

Fig. 4.—*A* is the microtessar of an infarct of twenty-four hours' duration. *B* is the microtessar of an infarct of seven days' duration. Note the sharp line of demarcation between the normal and the abnormal tissue and the preservation of the bronchi with the dilated bronchial vessels in the infarcted area. *C* is a photomicrograph of an area of infarction of twenty-four hours' duration. Red blood cells fill the alveoli, capillaries and the large blood vessels. *D* shows an area of infarction of two days' duration. The red blood cells are clumped and completely fill the alveoli. Many of the lining alveolar cells are not apparent.

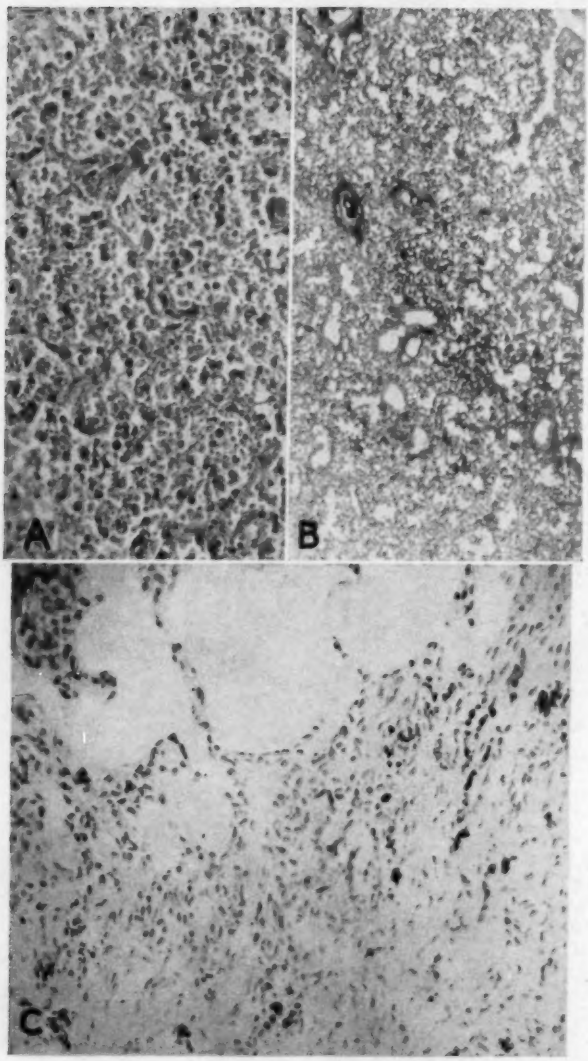

Fig. 5.—*A* shows an area of infarction of seven days' duration. There is some necrosis, but the red blood cells are fewer than in figure 4 *D*. There are several phagocytic cells. *B* is a photomicrograph of an infarct of two months' duration with interstitial fibrosis. *C* shows an infarct of six months' duration, with diffuse fibrosis involving the alveolar parenchyma.

necrosis and scar formation coincides with the hemorrhagic infarct encountered in human pathologic conditions and is evidently due to the assumption of some of the nourishment of the lung by the dilated bronchial circulation.

Another type of lesion observed coincided with roentgenograms of areas of the lung with partial obstruction of the pulmonary arteries and only very slight visualized bronchial circulation. The gross and microscopic pictures varied in degree of abnormality apparently with the extent of pulmonary obstruction. Grossly, the lesions were light red and occasionally pale pink, triangular (or irregular in shape if within the parenchyma), flat, with a slightly red, somewhat moist cut surface of normal consistency. Microscopically, the alveoli contained red blood cells in moderate numbers and there was some desquamation of the alveolar lining cells. The capillaries and the larger blood vessels were dilated and filled with blood. After several days, usually on the sixth, the areas were paler than the rest of the lung, and some of the vessels contained fibrin and hyaline thrombi. After two or three months there was slight patchy interstitial fibrosis.

The picture described apparently represents tissue changes in the lung due to partial closure of the pulmonary arterial blood supply. It is problematic whether such changes should be considered as true infarcts if complete closure of a supplying vessel is to be continued to be accepted as the genesis of an infarct.

SUMMARY

The introduction of a large number of emboli into the pulmonary arterial tree of a dog is not followed by any untoward symptoms nor is it incompatible with life, at least over one and one-half years, the maximum period of observation in these experiments. As much as 79 per cent of the dog's total lung by weight can be deprived of its pulmonary arterial circulation without causing death of the animal. The obstruction produced in these experiments was not of the main pulmonary trunk (as with other investigators) but of the branching tree of the arteries. As far as the dog is concerned, under the conditions of our experiments there is no justification for the concept that multiple emboli as such cause either immediate or delayed death by their presence in the lung or by a reflex.

Iodized oil injected into the pulmonary arteries of a living animal outlines the arterial tree and determines the areas of the lung to which the circulation is shut off. Apparently, because of the extensive vascularity of the lung, emboli do not close the blood supply unless they are introduced in fairly large numbers. A small number of emboli may, however, interfere in part with the circulation to a given area of the

lung. The mere introduction of foreign bodies into the pulmonary arteries should not lead to the assumption that an infarct must develop at the site of embolism.

The lung tissue deprived of its pulmonary blood supply shows some restoration after a period of three or more weeks. The pulmonary arteries in the areas of infarction become visualized on injection of iodized oil, and canalization and newly branching arteries become more profuse with time.

The bronchial circulation may be outlined by injection of iodized oil in a living animal. In a normal dog the bronchial arteries are very indistinctly outlined. In infarcted areas the bronchial vessels are well visualized, denoting dilatation. It is assumed that the dilated bronchial circulation is responsible for the failure of infarcts of the lung to proceed to complete necrosis and scar formation. The bronchial arteries dilate irrespective of the size, location or multiplicity of infarcted areas.

Grossly and microscopically all the criteria of hemorrhagic infarcts are present in the lungs of dogs with sufficient emboli to produce complete obstruction of the blood supply. No other factor except emboli is necessary for the production of infarcts. Partial interference with the pulmonary blood supply results in certain morbid changes consistent with the degree of obstruction.

PRODUCTION OF INTIMAL CHANGES IN THE ARTERIES

ATTEMPTED IN THE RAT BY PROLONGED FEEDING OF ACETO-ACETIC ACID

JAMES J. SHORT, M.D.

MAURICE BRUGER, M.D.

AND

LOUIS JAFFE, M.D.

NEW YORK

It is generally conceded that the paramount problem in the management of diabetes mellitus today is the prevention of arteriosclerosis, which manifests itself particularly in the coronary vessels and in the arteries of the lower extremities. The pathogenesis of vascular sclerosis has been discussed widely,[1] and there is no need at this time to detail any of the several theoretical considerations; suffice it to say that the theory of lipid infiltration (Anitchkov; Aschoff) has received the foremost attention. On the assumption that the primary manifestation in the atherosclerotic process is a loosening and swelling of the cement substance of the elastic fibers in the intima, it appeared important to learn whether an excess of acetone bodies in the blood, as frequently occurs in uncontrolled (and in controlled [2]) diabetes, is a factor in softening the intimal cement substance, thereby favoring the imbibition and deposition of lipids, particularly cholesterol esters. The present study was carried out to determine the effect of prolonged ketosis produced by the daily ingestion of aceto-acetic acid [3] on the histopathologic picture of some of the major arteries in rats.

Aided by grants from the Life Extension Institute and the Harriet Weil Fund.

From the Department of Medicine and the Department of Pathology and Bacteriology, the New York Post-Graduate Medical School and Hospital.

1. Cowdry, E. V.: Arteriosclerosis: A Survey of the Problem, New York, The Macmillan Company, 1933.

2. Short, J. J.: Unpublished studies.

3. Preparation of aceto-acetic acid: To 320 cc. of ethyl aceto-acetate in a 2 liter beaker surrounded by cracked ice was added 680 cc. of four times normal sodium hydroxide slowly and with constant stirring. The solution was allowed to stand for several hours (usually overnight) until equilibrium was reached. It was then washed three times with ether in a separatory funnel in order to remove any free ethyl aceto-acetate. The solution was then subjected to aeration in order to remove all traces of ether, the greater part of the alcohol resulting from the

(Footnote continued on next page)

EXPERIMENTAL METHOD

Twenty-eight rats from five litters were obtained, the ages ranging from 49 to 77 days. A uniform and adequate diet (Sherman diet 13) was fed to all the animals throughout the entire experiment. The preliminary period of observation lasted for approximately two months. Eight rats were then kept separate as controls; the remainder were given 500 mg. of aceto-acetic acid (calculated as sodium aceto-acetate [3]) daily with their water ration for a period of three months. During this time two of the experimental animals died. The eighteen surviving rats were then divided into three groups of six each; each rat in the first group was given 500 mg. of aceto-acetic acid daily; each rat in the second group, 1,500 mg., and each in the third group, 2,500 mg. The last dose proved toxic, and one month later, after four animals in this group had died, the two surviving animals were given 1,500 mg. daily until the end of the experiment. Autopsies were begun six months after the feeding of aceto-acetic acid was started and were continued at intervals until the end of the study, approximately one year after its initiation. No pathologic examination was made of the arteries of any animal found dead in its cage, thereby eliminating the factor of postmortem arterial changes.

The animals were weighed at intervals of one week. The control rats appeared to be healthy, and they gained weight consistently. The experimental animals, however, lacked vigor; they appeared listless and showed a consistent loss of weight. Four months after the feeding of acid was begun it was noted that the fur on the lower portion of the abdomen of the experimental rats was turning brown. This discoloration persisted throughout the entire experiment.

It appeared important to know whether definite acetonemia was produced by the feeding of aceto-acetic acid; the possibility of prompt oxidation of the acetone bodies with the use of a normal diet was considered. Since sufficient blood for chemical analysis could not be obtained without killing the animal,[4] a test was made for the presence of acetone in the expired air. This was done by placing the rat in a small box through which a current of air was passed. The air was then bubbled through the Scott-Wilson reagent; in the presence of acetone, the reagent assumed a milklike turbidity. Several such tests were carried out throughout the course of the study; in every instance each of the experimental animals showed the presence of acetone in the expired air, while the control rats showed none.

Pathologic Technic.—The following technic, suggested by Dr. Ward J. MacNeal, was used in order to fix and study the arteries in a state of distention closely approximating normal. By means of a closed hydraulic system the air pressure

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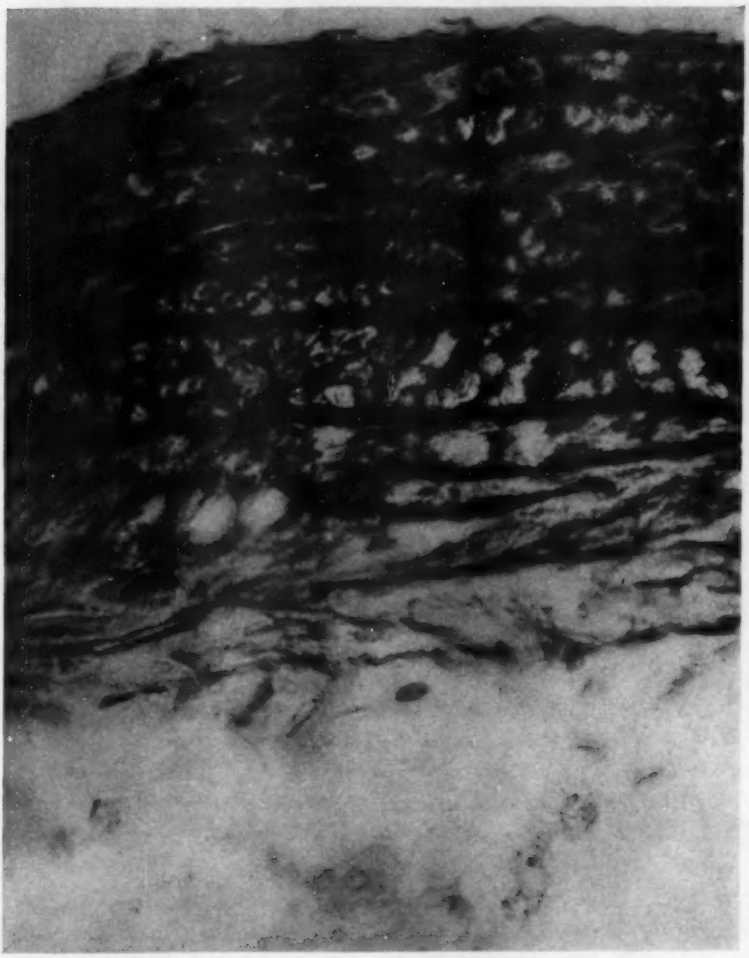
saponification of the ethyl ester and most of the acetone resulting from the breakdown of any aceto-acetic acid present (Butts, J. S., and Deuel, H. J., Jr.: J. Biol. Chem. **100**:415, 1933. Butts, J. S.: Personal communication to the authors). The concentration of sodium aceto-acetate present in the solution was then determined according to the method for total acetone bodies described by Van Slyke and Fitz (J. Biol. Chem. **32**:495, 1917; **39**:23, 1919), which yielded approximately 50 per cent. Immediately before feeding the solution to the rats a known quantity of sodium aceto-acetate was made slightly acid by the addition of a 10 per cent solution of hydrochloric acid, bromthymol blue being used as an indicator.

4. Two or three cubic centimeters of blood from each of three experimental animals was analyzed for total acetone bodies according to the method of Van Slyke and Fitz. Although the values are not reliable, because of the small quantity of blood used, the amount of precipitate obtained suggested the presence of acetonemia. The blood from three control animals was free from acetone bodies.

was obtained by water displacement. This pressure was then led to two carboys and was used to displace physiologic solution of sodium chloride and Helly's solution (Zenker's solution to which a dilute solution of formaldehyde has been added), respectively. A control and an experimental rat were chloroformed, and the thoracic aorta was exposed. A cannula was then inserted in the direction



Photomicrograph showing a large atheromatous plaque of the aorta. No normal areas are visible. Van Gieson elastica stain; × 470.

of the blood flow, and the vascular system was perfused first with physiologic solution of sodium chloride and then with Helly's solution for fixation. As the entire perfusion apparatus was a closed system, a manometer attached to the compressed air gave a fairly accurate index of the intra-aortic pressure, which was maintained at 65 mm. of mercury by regulation of the inflow of water. Perfusion

with the fixing fluid was maintained for from six to eight hours, so that the arteries were fixed in the systolic phase. Sections for study were then taken from the aorta and the iliac, femoral and tibial arteries.

RESULTS

Fourteen rats came to autopsy, six of which were controls. No pathologic lesions were observed in the arteries of the six control animals; only one experimental animal showed intimal lesions in the aorta and the femoral artery (fig. 1). This rat had received 500 mg. of aceto-acetic acid daily for three months, followed by 1,500 mg. daily for approximately six months. Figure 1 shows a heaping up of the intima at various points similar to the projection into the lumen of the atheromatous cushion. In the cross-section the circular elastic fibers, which are ordinarily arranged into from four to six concentric layers, are broken up and split into many more layers, forming a dense fibrillar network in which the predominating direction of the fibers is still circular. The matrix or cement substance between the elastic fibers appears to be greatly altered and contains many vacuolar spaces as well as irregularly distributed areas of granular material. There is an associated diffuse cellular hyperplasia. While these changes extend almost to the adventitia, the intimal areas show the most marked degree of involvement and are uniformly thickened. The intimal thickening, evidence of inflammation and disturbance in the cement substance are seen essentially in the atheromatous lesion of recent origin. Whether or not the vacuolar areas contained lipoid bodies, as observed in early atheroma, can only be conjectured.

COMMENT

Since only one of the eight surviving experimental rats showed the lesions just described, it appears safe to say that prolonged ketosis produced by the daily feeding of aceto-acetic acid does not produce alterations in the histopathologic picture of the arterial wall in rats. It is possible that the animal showing the lesions indicated in figure 1 manifested a spontaneous change, such as was recently described by Hueper [5] in the pulmonary artery in rats.

The problem, however, demands more intensive study before any definite conclusion can be drawn; this is true especially when the results of such experimentation are applied to human beings in the diabetic state. The ketosis of diabetes mellitus is probably associated with a more profound, though perhaps more variable, acetonemia, and again the quantitative relationship of the various ketone bodies in diabetic acidosis is undoubtedly different from that which can be produced under the conditions of the present experiment. For these reasons the pancreatectomized animal appears to be an ideal medium for the study of the effect of prolonged diabetic ketosis on the arterial wall. Unfortunately, additional factors invariably accompanying the acidosis of

5. Hueper, W. C.: Arch. Path. **20**:708, 1935.

diabetes, such as hypercholesteremia, hyperglycemia and dehydration, each of which may be instrumental in producing intimal damage, would soon confuse the issue. The present experiments were performed to overcome these difficulties; the results, as far as aceto-acetic acid is concerned, may therefore be significant.

CONCLUSION

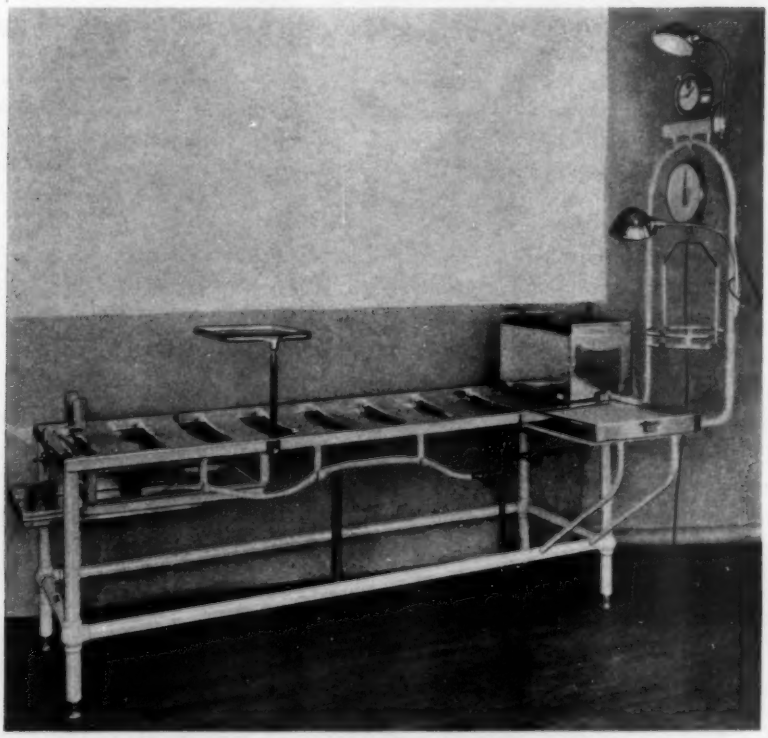
An attempt has been made to produce intimal changes in some of the major arteries in the rat by the feeding of aceto-acetic acid. The results indicate that under the conditions of these experiments prolonged ketosis is probably not a factor in altering the structure of the intima.

Laboratory Methods and Technical Notes

AN AUTOPSY TABLE OF COMPACT AND CONVENIENT DESIGN

K. V. Kitzmiller, M.D., Cincinnati

The autopsy table [1] shown in the accompanying illustration was designed to save unnecessary steps and to provide all of the facilities usually needed in making postmortem examinations.



An autopsy table that provides ideal working conditions.

The top and all working parts are made of stainless steel and are supported by a painted heavy metal base.

There is a large metal wash basin with running water mounted at the foot, while movable transverse metal slabs keep the body from soiling and permit water to flow over the bottom of the table from each end to a central drain pipe connected with the sewer.

From the Pathological Laboratory, St. Mary Hospital.

1. Built by Max Wocher & Son Co., Cincinnati.

A hose is also provided for general washing and cleaning purposes.

The most convenient part of the table is a right-angled extension at the foot, suitably elevated to permit drainage and having an inlaid cork base for cutting organs and tissues.

A steel tape is attached to one side of this extension, while immediately above is a spring scale pan with steel barriers to prevent slipping of the organs while being weighed. An electric clock is mounted above, and the scale dial is illuminated by an adjustable goose neck light. There is also a light for illumination of the table. A movable Mayo instrument tray is attached to one side of the table.

At the head is an adjustable wooden rest, while beneath is a stainless steel pull-out tray of use in necropsy of the head. A flexible goose neck light (not shown in the illustration) is also attached here.

This table has been in use for over two years, and the ideal working conditions which it offers makes it worthy of report.

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, etc.—The John Scott Award of the City of Philadelphia has been given to James Ewing for his work on tumors.

Joseph McFarland has retired as professor of pathology in the University of Pennsylvania.

Karl Pearson, the mathematician who placed the methods of medical statistics on a sound basis, has died in his eightieth year.

The retirement is announced of Leo Loeb, professor of pathology in Washington University, St. Louis. Howard A. McCordock, associate professor of pathology in the same university, has been appointed to succeed Dr. Loeb.

Friedrich Breinl, professor in the University of Praha, died on July 29, 1936, from Rocky Mountain spotted fever, with the virus of which he had been working for some time.

Henry Sewell, pioneer American physiologist, who demonstrated that pigeons could be immunized against rattlesnake venom in 1887, died on July 8, 1936, at the age of 81 years.

The Marcel Benoit Prize of 30,000 francs has been awarded to M. Askanazy, Geneva, Switzerland, for his researches on cancer.

The National University of Ireland has conferred the honorary degree of doctor of science on Simon Flexner, emeritus director of the Rockefeller Institute for Medical Research, New York City.

William D. Collier, professor of pathology in St. Louis University, has accepted the headship of the department of pathology of St. Elizabeth's Hospital, Youngstown, Ohio, succeeding Robert B. Poling.

Ernest Witebsky has been appointed associate professor of bacteriology and immunology in the University of Buffalo and bacteriologist to the Buffalo General Hospital, Buffalo.

Wiley D. Forbus, professor of pathology in Duke University, Durham, N. C., has been elected a member of the National Board of Medical Examiners, succeeding Howard T. Karsner.

Etienne Burnet has been appointed director of the Pasteur Institute of Tunis, in succession to the late Charles Nicolle.

In Tufts College Medical School, Boston, John L. Jacobs has been appointed associate professor of pathology and bacteriology.

Robert P. Moorehead has been elected instructor in pathology in the school of medicine of Wake Forest College.

The American Board of Pathology.—The board has been organized under the auspices of the American Medical Association, with A. H. Sanford as president and F. W. Hartman (Ford Hospital, Detroit) as secretary-treasurer, to whom all communications should be addressed. There are six other members. The purposes of the board are to advance the science and practice of pathology and to issue certificates to applicants who voluntarily comply with its requirements. Applicants with certain special qualifications may be certified without examination up to July 1, 1938.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Physiology

Nutritional Edema in the Dog. A. A. Weech, E. Goettsch and E. B. Reeves, Bull. Johns Hopkins Hosp. **58**:1, 1936.

Experiments are described in which the intake and output of sodium chloride were followed (*a*) in a normal dog and (*b*) in animals during the progressive depletion of the serum albumin which follows maintenance on a diet low in protein. The findings, summarized at the conclusion of part I of this paper, warrant the inference that canine and human subjects respond in a similar manner to the administration of salt. Further experiments are described in which an attempt was made to discover why ingestion of salt and water leads to augmentation of fluid in the interstitial reservoir. When salt is administered after a period of salt deprivation one consistently finds after a period of about eighteen hours that the specific gravity and total protein of the serum and the relative red cell volume of the blood have lower values than initially. Calculation indicates an average decline in the colloid osmotic pressure of the serum of 17 or 20 mm. of water. The direct measurement of plasma volume suggests that the recorded changes in gravity, protein and hematocrit reading result from an increase in the volume of the plasma. Because of the difficulty of measuring plasma volume with accuracy, the individual findings are thought to be devoid of quantitative value, and it is suggested that a better approximation of the magnitude of the increment in plasma volume is obtained by calculations based on the changes in protein, gravity and hematocrit reading. Such computations indicate an average increase in plasma volume of about 10 per cent. The possibility is suggested that the increase may be associated with a rise in capillary blood pressure. A discussion is given of the part played by the kidney in regulating the distribution of fluid in the body and of the rôle of this organ in determining the findings in this and in previous investigations.

From the Authors' Summary.

Transplantation of Splenic Tissue in Mice. J. J. Bittner, Pub. Health Rep. **51**:244, 1936.

Retention of implants of splenic tissue and probably of all normal tissue is dependent on the simultaneous presence in the genetic make-up of the host of all the growth factors found in the genetic constitution of the graft.

From the Author's Conclusion.

Effect of Renal Denervation on the Blood Pressure in Experimental Renal Hypertension. W. M. Arnott and R. J. Kellar, J. Path. & Bact. **42**:141, 1936.

A progressive fall in blood pressure follows bilateral nephrectomy in the rabbit. The hypertension in oxalate nephritis appears to be due to renal damage. Denervation of the kidney abolishes the hypertension associated with oxalate nephritis in unilaterally nephrectomized rabbits.

From the Authors' Conclusions.

HEMOCHROMATOSIS AND HEREDITY. R. D. LAWRENCE, Lancet **2**:1055, 1935.

In a family of nine two brothers had typical hemochromatosis; three other brothers and the mother had signs of the disease, but the sisters were free from all such signs. Lawrence suggests that the observation concerns a hereditarily sex-linked error in iron metabolism with a gradual accumulation of iron pigments that finally results in the clinical manifestations of the disease.

BONE CHANGES IN CHICKENS WITH ARTIFICIALLY PRODUCED OSTEITIS FIBROSA. W. GOHS, Frankfurt. Ztschr. f. Path. **47**:63, 1935.

Gohs injected into chickens glycerin extracts of bone and marrow from young growing chickens which had been previously exposed to roentgen radiation. As a result of this treatment he observed in a number of the animals changes in the bones resembling osteomalacia and rickets in addition to fibrous osteitis. It was possible to produce in young, growing chickens within a few months a disease of the bones of the Recklinghausen type. The temporary cysts and tissue-like giant cell tumor, mainly about the metaphysis, were also observed. In adult chickens similar changes developed but more slowly, less extensively and usually without the formation of giant cell tumor-like tissue and cysts. After destruction of the intermediary cartilage in the young chickens, with resulting termination of bone growth, the course of the disease in young and old chickens was identical.

In the first stages of the disease new production of bone was more pronounced than destruction of bone. In the later stages in one group of animals destruction prevailed; in another group the two processes were in equilibrium. Healing occurred with the final production of a somewhat irregular hard dense bone of mosaic appearance and thickening of the cortical zones.

In chickens hatched in the fall and kept in indoor coops Gohs observed, in the absence of all treatment, changes similar to rickets. On being treated with the injections mentioned these chickens revealed bone changes which may be classified as transitional from rickets to fibrous osteitis.

OTTO SAPHIR.

THE HYPERERGIC INFLAMMATORY REACTION OF DENERVATED TISSUE. D. N. WYROPAJEW, Virchows Arch. f. path. Anat. **295**:65, 1935.

In rabbits one hind limb was denervated by section of the sciatic and femoral nerves and by application of solution of formaldehyde U. S. P. to the adventitia of the vessels of the hip region. The opposite limb served as a control. The animals were sensitized by subcutaneous injection of horse serum. At varying intervals after denervation the reaction of the skin to the local injection of the serum and that of the muscle after the injection into it of the activating dose of serum were studied histologically. During the period from the first to the fifth day after denervation the reaction of the denervated muscle was the same as or even more intense than that of the control. During the period from the sixth to the tenth day a decided decrease in the intensity of the reaction was evident in the denervated muscle, the lesser response being quantitative in relation to the time after denervation. During the period from the tenth to the thirtieth day a characteristic hyperergic reaction could no longer be evoked; the inflammatory process was of the normergic type. After thirty days, when trophic changes were evident in the muscle, the inflammatory reaction was less marked than in the control muscle and was of the anergic type. The skin of the limb operated on yielded at all stages a hyperergic response identical with that of the control limb, indicating that the operative procedure had had little effect on the innervation of the skin. The author concluded from his experiments that the local nervous system plays a decisively important rôle in the development of the hyperergic inflammatory reaction.

O. T. SCHULTZ.

Pathologic Anatomy

THE PATHOGENESIS OF LIPOID NEPHROSIS AND PROGRESSIVE GLOMERULONEPHRITIS. S. S. BLACKMAN JR., Bull. Johns Hopkins Hosp. **57**:70, 1935.

A case of chronic glomerulonephritis which resembled lipoid nephrosis in practically every particular is described. There was massive edema. The urine contained large amounts of protein, many casts, doubly refractive lipoid crystals, leukocytes and very few red blood cells. The plasma protein content was low; the albumin-globulin ratio was inverted. The blood cholesterol content was very high. At autopsy the kidneys were large, smooth and yellow, without gross scars. There was microscopic evidence of damaged tubular and glomerular epithelium, and lipoid deposits were plentiful. There is evidence to suggest that chronic pneumococcic infection may be concerned in the pathogenesis of certain cases of chronic glomerulonephritis. FROM THE AUTHOR'S SUMMARY.

SYPHILIS OF THE MITRAL VALVE AND MEMBRANOUS INTERVENTRICULAR SEPTUM OF THE HEART. S. S. BLACKMAN JR., Bull. Johns Hopkins Hosp. **57**:111, 1935.

Here are described two examples of syphilis of the mitral valve and membranous interventricular septum occurring with aortic insufficiency and typical syphilitic lesions of the aortic valve and aorta. The lesions in the membranous septum and mitral valve were directly continuous with the syphilitic changes in the root of the aorta and aortic valves. The body of the anterior segment of the mitral valve was therefore chiefly affected, and the posterior mitral leaflet was relatively unaltered. The gross lesions consisted of diffuse leathery thickening of the membranous septum and great anterior mitral leaf. The left ventricular surface of the affected mitral segment and septum showed wrinkling and puckering resembling typical gross syphilitic lesions of the aorta. The mitral chordae tendineae were slightly affected. Microscopically, gummatous necroses or dense vascular scars with perivascular round cell inflammation, or both, were found in the middle layers of the membranous septum and the mitral valve. These lesions, continuous with the syphilitic lesions in the media of the aorta, were overlaid by strata of scar tissue formed especially on the left ventricular surface of the valve and septum. Scars and perivascular inflammation were found in the wall of the left auricle and in the muscular interventricular septum near the insertion of the syphilitic mitral valve and membranous septum. Mitral lesions were suspected clinically in both of the cases described. Each case was characterized by persistent cardiac decompensation which could not be relieved, lasting for five and a half months in one case with extreme aortic insufficiency, and for a year and a half in the other, in which the aortic valve was moderately insufficient. The anatomic changes in the mitral valve in each case suggested some degree of insufficiency, and it is probable that the mitral lesions were in part responsible for the degree and persistent character of the cardiac decompensation. FROM THE AUTHOR'S SUMMARY.

ENDOMETRIAL STUDIES. G. S. MCCLELLAN, D. PHELPS and J. C. BURCH, Endocrinology **19**:321, 1935.

These studies have demonstrated that the use of the punch to secure tissue for biopsy is a reliable and safe method of obtaining endometrium for clinical and research purposes. Proper use of the data reported will simplify the diagnosis of endometrial hyperplasia and do much to differentiate luteinic from aluteinic bleeding. It has been clearly shown that the so-called Swiss cheese pattern is not a constant accompaniment of endometrial hyperplasia and that the diagnosis can and should be made in its absence. In the case reported, bleeding followed a decline in the level of estrogenic hormone in the blood and was checked following a rise in this level. The reactions of human endometrium to injections of estrogenic hormone and progestin are essentially the same as those of the endometrium of the lower

animals. A dose of 250,000 mouse units provoked a mild growth of the endometrium. The cessation of the administration of the estrogenic hormone was followed by bleeding.

XANTHIC LESIONS. M. M. MELICOW, J. A. M. A. **105**:768, 1935.

The reticulo-endothelial system is important as an entity and as a mechanism responding to accumulations or excess of lipoids and particulate matter. Accumulations of lipoids may result from inflammatory, traumatic or deficiency causes. The lipophagic response by cells of the reticulo-endothelial system results in the formation of a xanthic mass. The cell unit of the xanthic mass is the foam cell. The foaminess results from the solution of cell lipoids during the routine process of preparation. Should scarlet red be used, the lipoids appear as red intracellular droplets. The orange color of a xanthic mass is due mainly to a combination of its lipoids and lipoid pigment content and to a lesser degree to its blood pigment. An outline of xanthic entities which has been included embraces the xanthic granulomas, the xanthic lipoidoses and the so-called xanthoma and calls attention to lesions that resemble them in color but are basically different. In two cases, unusually extensive "xanthomatosis" of the kidney was present, and in one case a seemingly similar condition was found in a carcinoma of the prostate.

FROM THE AUTHOR'S SUMMARY.

EAR EXOSTOSES. A. HRDLIČKA, Smithsonian Misc. Collect., vol. 93, no. 6, 1935.

Exostoses of the ear are localized hyperplasias, or outgrowths from essentially the tympanic part, but occasionally also from the squamous portion, of the external bony meatus. They arise generally from what were the free upper ends of the tympanic ring. Their development belongs chiefly to the later adolescent period and the earlier half of the adult period. A predisposition to exostoses of the ear is now probably universal in man, but in some races or groups the formation of the abnormalities, owing perhaps to direct hereditary effects, is much more frequent than in others. Males are considerably more subject to the growths than females, the well-to-do (among the whites at least) more than the poor. The abnormality is most frequently bilateral; when one-sided, it occurs somewhat more commonly in the left than in the right ear. Structurally, the growths range from cancellous to compact, without any definite segregation. Though macroscopically the bone is often more or less aberrant, its elements are normal and remain viable. There is never any bréaking down or necrosis, nor complete calcification. The causes are systemic or predisposing and exciting. The paramount systemic cause appears to be a deranged neurovascular control of the parts involved, chiefly the tympanic bone, during what corresponds to the sexually more active part of life; but on critical consideration it becomes apparent that no connection of the exostoses with sex activity can be ascertained. What causes the peculiar time-limited neurovascular derangement that predisposes or leads to exostoses of the ear cannot yet be definitely established, but it appears to be something in the hereditary endowment of the trophic nerve centers that control the normal status of the external bony meatus. A deranged accommodation of evolutionary nature suggests itself, rather than degeneracy, as a plausible explanation. The exciting cause of such exostoses, when the predisposition to these exists, may be anything mechanical or chemical that produces prolonged irritation, with consequent hyperemia progressing to inflammation, of any part of the bony meatus.

FROM THE AUTHOR'S SUMMARY.

COMPLETELY HEALED PERIARTERITIS NODOSA. B. KNAUER, Centralbl. f. allg. Path. u. path. Anat. **63**:161, 1935.

Knauer believes he has found the fully healed lesions of periarteritis in the kidneys of a man 50 years old who died of pulmonary tuberculosis. There were marked evidences of atherosclerosis in all the organs. White scars, varying in size

up to that of linseed, occurred in the renal cortices and extended to the medullas. Histologically, these nodules consisted of connective tissue, at times loose, at others compact, surrounding atrophic uriniferous tubules, glomeruli in various stages of fibrous replacement and markedly altered blood vessels. The vascular changes were most pronounced in the arteriae rectae and less evident in the veins and arteriae arcuatae. These changes consisted of outpouchings, diffuse or circumscribed, in which the media was markedly thinned or entirely lost and the internal elastic membrane stretched and thickened up to fourteen times the normal. The intima was often thickened to compensate for defects in other layers and frequently formed thick ridges almost occluding the lumen.

GEORGE RUKSTINAT.

THE BLOOD SUPPLY OF THE SENILE HEART. H. SAGEBIEL, Virchows Arch. f. path. Anat. **294**:147, 1934.

The blood supply of the heart, as determined by morphologic study of the coronary arteries, was investigated in fifteen hearts from persons aged from 70 to 90 years. Seven additional hearts from persons aged from 40 to 65 years were used as controls. A mixture of barium sulfate and gelatin was injected into the coronary arteries at a pressure of 180 mm. of mercury. After fixation, blocks for making frozen sections were taken from the left anterior descending artery, the circumflex and the right coronary artery at distances of 2 and 5 cm. from the origin of the arteries. On sections the surface area of the lumen and of the entire vessel (lumen, intima and media) was determined by measurement. The hearts used were grossly normal. In the senile group a decrease in the weight of the heart was not observed. In relation to body length the cardiac weight remained constant. In relation to body weight the cardiac weight increased with age, owing to the senile atrophy of the body tissues. The surface area of the lumen of the coronary artery remained constant, whereas the surface cross-sectional area of the vessel increased progressively with age, owing to thickening of the intima and media. The diameter of the lumen is dependent on the size and weight of the heart. The progressive thickening of the walls of the coronary arteries with advancing years causes no decrease in the width of the lumen.

O. T. SCHULTZ.

STUDY OF THE OSSEOUS SYSTEM IN A CASE OF HAND-SCHÜLLER-CHRISTIAN'S DISEASE. G. GERSTEL, Virchows Arch. f. path. Anat. **294**:278, 1934.

Concerning the nature of the lipoid infiltrative disease to which the names of Schüller and Christian are usually attached but which Hand described first, two main views obtain. The first, which is the predominating one, is that the condition is primarily a disturbance of lipoid metabolism, to which the deposit of lipoid-infiltrated tissues especially in the skull, ribs and vertebrae is secondary. According to the second view, proposed by Ighenti in 1931, the disease is primarily a generalized granuloma in which cholesterol infiltration is a secondary phenomenon. The case which forms the basis of Gerstel's detailed and careful study was that of a girl aged 2 years and 4 months at the time of death. Evidences of the disease had been present for four and one-half months before death. The entire skeleton was submitted to gross and microscopic examination. Granulomatous lesions were observed in all the bones, even when no macroscopic changes were evident. The lesions were smallest in the smallest bones, such as those of the digits, and increased progressively with the size of the bone. The granulomatous tissue caused lacunar resorption of the bone. Similar lesions, usually of miliary size, were present in the spleen, liver, lungs, lymph nodes, intestine and skin. The smallest miliary lesions, in both bones and soft tissues, were perivascular. They did not contain lipoid material. As the lesions became larger histiocytes filled with lipoids appeared. In still older lesions evidences of active cellular proliferation were absent, and the lesions consisted of lipoid-filled foam cells. Chemical examination revealed the

increased cholesterol content and the disturbed ratio of cholesterol to cholesterol ester characteristic of the disease under consideration. Gerstel concludes that the disease is primarily a generalized granulomatosis, of unknown origin that widely involves the skeleton and soft tissues. Cholesterol infiltration of the granulomatous tissue is a secondary process. The cause of the hypercholesteremia and cholesterol infiltration is as yet unknown.

O. T. SCHULTZ.

Microbiology and Parasitology

EXPERIMENTAL CHRONIC CUTANEOUS BLASTOMYCOSIS IN MONKEYS. W. A. DEMONBREUN, Arch. Dermat. & Syph. **31**:831, 1935.

From a case of facial blastomycosis of nine years' duration in a farmer aged 60 an organism was isolated and identified as Monosporium tularense (Castellani). This organism may be cultivated either in the yeastlike form or as mycelium. Lesions were induced in three monkeys by subcutaneous and intradermal injections of cultures of the fungus and were similar to those seen in man. The blastomycosis in the monkeys tended to become systemic. In the pathogenic phase the fungus is a chlamydospore which germinates in tissues by budding and appears as a yeastlike form. This is an excellent article on blastomycosis.

S. W. BECKER.

THE PRIMARY COMPLEX OF TUBERCULOSIS OF THE SKIN. H. E. MICHELSON, Arch. Dermat. & Syph. **32**:589, 1935.

Michelson reports two cases of tuberculosis from primary intracutaneous inoculation. In the first case the lesion developed on the ball of the right index finger of a middle-aged woman following injury by a splinter from a drinking glass; in the second the lesion developed on the right ankle of a boy aged 18. The true primary complex of tuberculosis of the skin occurs in nontuberculous persons and therefore usually in children. The lesion usually follows injury and is a papule and later an ulcer. This is followed in from three to four weeks by lymphangitis and regional adenitis. The initial lesion tends to heal with a scar; there may be a residual tuberculous infiltrate beneath a superficial firm scar. The adenitis usually involves the nearest draining gland first. By the time the diagnosis is made the reaction to tuberculin is usually positive, and it has been reported positive as early as one week after the injury. The patient should have had a negative reaction to tuberculin previously.

S. W. BECKER.

SUMMARY OF FIFTY-THREE REPORTED CASES OF RHINOSPORIDIOSIS. W. A. E. KARUNARATNE, J. Path. & Bact. **42**:193, 1936.

Most of the patients have been observed in India (forty); a few, in Ceylon (four), North America (five), South America (three) and Italy (one). The very wide distribution of the disease is noteworthy. In the solitary European case (Orlandi) a rhinosporidial growth developed in the conjunctiva of a woman aged 56 following injury of the eye by a splinter. Seeber's patient, whose case was the first reported, was also a native of Italy but had been a resident in South America since his infancy. The case described by Ashworth occurred in an Indian student who had come to Edinburgh to study, and the two other cases referred to in his monograph also occurred in Indians, as did the cases described by Beattie and by Shrewsbury. Of the four cases reported from Ceylon, three were observed in persons who had not been out of the island. With my thirty-four cases, the total number occurring in Ceylon is only a little short of the total number of Indian cases. All five cases reported from North America occurred in widely separated areas. Only one of the patients had been outside the United States and that was for a three day visit to Canada. Wright's patient had lived all his life in the neighborhood of Memphis, Tenn., while Graham's patient, a 12 year old

Negro boy, born in Georgia, had resided in a small coal-mining village in Alabama since he was 4. The ages, recorded in thirty-three cases only, ranged from 8 to 60 years. The disease mainly affects young persons, twenty-four of the thirty-three subjects being below the age of 30 and twelve below the age of 20. Only four of the fifty-three patients were females. In forty of the fifty-three patients the lesion was in the nose, mainly in the anterior part. The conjunctiva was affected in eight, the lacrimal sac in two and the penis, uvula and external auditory meatus each in one. A woman had a growth in the nose and an independent growth on the posterior pillar of the fauces. In my own series of thirty-four cases the condition was conjunctival in two, lacrimal in one and nasal in the rest.

FROM THE AUTHOR'S SUMMARY.

THE INTESTINAL CHANGES FROM HEMATOGENOUS PARATYPHOID INFECTION IN RABBITS. ALICE WALDMANN, Zentralbl. f. Bakt. (Abt. 1) **133**:196, 1935.

Living paratyphoid bacilli (Breslau type) were injected intravenously into rabbits to determine whether or not they are eliminated through the intestinal wall or excreted in the bile. Twenty-one of twenty-four rabbits which were killed from six to eight days after the intravenous infection of the bacilli showed definite inflammatory changes in the lymphatic apparatus of the intestine. The lymphoid follicles contained in their centers sharply circumscribed necrotic nodules or showed accumulations of leukocytes or proliferations of epithelioid cells. These changes were not so extensive, however, as those seen in rabbits infected enterally. Furthermore, inflammatory changes in the mesenteric lymph nodes, which occur regularly in rabbits enterally infected, were seen only rarely in those with hematogenous infection and then were slight. On the other hand, purulent cholecystitis, which was extremely rare in animals enterally infected, occurred in about one fourth of the animals infected hematogenously. Severe illness, with definite ulceration of Peyer's patches and of mesenteric lymph nodes, resembling typhoid fever in human beings, occurred only following enteral infection. Waldmann concludes, therefore, that the intestinal lesions accompanying hematogenous infection with the Breslau bacilli represent the effects of excretion of the bacilli. The lesions, however, do not indicate a pathogenesis analogous to that of typhoid fever in the human being.

PAUL R. CANNON.

Immunology

LOCAL SKIN REACTIVITY TO BACTERIAL FILTRATES. G. SHWARTZMAN, J. Exper. Med. **62**:621, 1935.

The skin of the rabbit's ear is considerably more resistant than the abdominal skin to the phenomenon of local skin reactivity to bacterial filtrates. Ten times the provocative dose is required if the filtrate injected is into the vein of the prepared ear and thirty times the provocative dose if into the vein of the non-prepared ear. The state of reactivity cannot be elicited by a preparatory intravenous injection of bacterial filtrate alone into the clamped or the nonclamped ear. The state also fails to appear when the injection is made in combination with the employment of cold, xylene, ethyl urethane, pilocarpine hydrochloride, atropine, calcium gluconate, extract of guinea-pig liver, histamine di-hydrochloride, epinephrine chloride and posterior pituitary. Preparatory intravenous injections of toxins are capable of eliciting the state of reactivity in the rabbit's ear when they are accompanied by the production of thermal hyperemia (i. e., exposure to 45, 50 and 55 C.). It is also possible to induce the state of reactivity when a mixture of the preparatory factors with testicular extract is injected into the veins of clamped ears. The period of incubation required may be less than two hours. In the light of these experiments, there are discussed various observations concerning the elicitation of the phenomena of organ reactivity by means of live bacterial cultures and the filtrates thereof.

FROM THE AUTHOR'S SUMMARY.

INCIDENCE OF SEROLOGIC TYPES OF HEMOLYTIC STREPTOCOCCI IN RECENT CASES OF ERYSIPELAS. S. SPICER, M. F. GONSHOREK and E. L. SPICER, J. Immunol. **28**:399, 1935.

From a study of sixteen strains of hemolytic streptococci of the beta type that were isolated from fifty-two cases of erysipelas, and from absorption and protective tests with homologous antiserums and with serums produced by a commercial firm and by the New York City department of health, it was concluded that antierysipelas immune serums are frequently deficient in antibacterial properties with consequent low protective value. In some cases, a striking interaction was observed between erysipelas and scarlet fever strains.

I. DAVIDSOHN.

EFFECT OF METHODS OF INJECTION ON THE SPECIES SPECIFICITY OF SERUM PRECIPITINS. H. R. WOLFE, J. Immunol. **29**:1, 1935.

Wolfe obtained highly species-specific precipitins by inoculating rabbits with a total of 3 cc. of serum divided into three doses, one given every other day. The highest titer was reached from ten to fifteen days after the last injection. The precipitin reactions with the serums of distinctly related animals were less than 2 per cent of those with the homologous antigen; the reactions with the serums of animals belonging to the same order varied from 2 to 100 per cent. Quantitative gradations of the precipitin reactions within an order made it possible to distinguish between families and even genera. Another method that employed five or more intravenous injections beginning with 1 cc. and increasing by 1 cc. at each successive injection was less satisfactory, owing mainly to a lower degree of species specificity. The reactions with the serums of very distantly related animals ranged from 2 to 25 per cent of those with the homologous antigen, and a differentiation between families and genera of the same order was not possible. When a second series of injections was administered, a loss of specificity was the result.

I. DAVIDSOHN.

THE MECHANISM OF IMMUNITY TO FILTRABLE VIRUSES. A. B. SABIN, Brit. J. Exper. Path. **16**:158 and 169, 1935.

Rôle of Leukocytes in Vaccinia.—Fresh normal rabbit serum and leukocytes are not viricidal for vaccinia. In the presence of normal serum, leukocytes fix the virus but become and remain highly infectious thereby. Leukocytes also fix virus in the presence of immune serum, but no evidence was obtained that the virus was subsequently destroyed. Leukocytes can fix the protective substance in immune serum in much the same manner as minced testis. Virus incubated with immune serum and ultracentrifugated is fixed apparently to the same extent, and the leukocytes remain as highly infectious as when virus which has been ultracentrifugated after incubation with normal serum is used. While the whole blood of an immune rabbit given intravenous injections of vaccinia virus is not infectious, the leukocytes were shown to contain active virus even twenty-four hours after inoculation. There is, therefore, no evidence that the immune serum exerts an opsonic action on vaccinia virus or that the leukocytes can destroy the virus in its presence.

Varying Protection by Antiviral Serum in Different Tissues.—It appears to be quite definite that in vivo, though tissues of equal susceptibility and equal amounts of immune serum and virus are employed, the outcome is not the same. The mechanism whereby one tissue is readily protected while another is not is obscure, and its elucidation is of the utmost importance to a clear understanding of the nature of immunity to filtrable viruses.

FROM THE AUTHOR'S SUMMARIES.

HEMOLYSIN FROM A STRAIN OF ANIMAL STREPTOCOCCI. H. LOEWENTHAL and M. G. PRADHAN, Brit. J. Exper. Path. **16**:230, 1935.

A method is described for the preparation, in a serum-free medium, of hemolysin from streptococci derived from an infected animal. The medium consists of tryptic

bicarbonate broth to which cysteine hydrochloride is added. The negative oxidation-reduction potential maintained in this medium during the whole period of incubation and filtration allows the secretion and harvesting of a potent streptolysin. The serum-free hemolysin of this animal strain is subject to reversible oxidation and reduction. Hemolysin produced by this strain in serum broth is not readily oxidized, and when the titer has dropped during storage it can be raised by reduction.

FROM THE AUTHORS' SUMMARY.

BLOOD GROUPS AND HEREDITY. N. P. MITRA, Indian J. M. Research **22**:495, 1935.

Two thousand persons from Dibragarsh (Assam, India) were examined, and the following distribution of the blood groups was found: group 0, 33.65 per cent; group A, 24.55 per cent; group B, 32.55 per cent; group AB, 9.25 per cent. The calculated frequency of group AB according to the triple allelomorph theory was 9.12 per cent, so that these results support Bernstein's theory.

A. S. WIENER.

THE PRECIPITIN REACTION: EXPERIMENTS ON MULTIPLE ZONES. G. L. TAYLOR and M. E. ADAIR, J. Hyg. **35**:169, 1935.

An antiserum containing antibodies for two unrelated antigens, crystalline egg albumin and crystalline horse serum albumin, has been prepared. In titrations of the antiserum against mixtures of these antigens it has been possible to produce a single zone or two zones of optimal particulation by varying the relative concentrations of the components in the antigenic mixtures. It is evident, therefore, that while (*a*) a single zone does not necessarily indicate a single antigen-antibody system, (*b*) multiple zones suggest the presence of more than one antigen-antibody system.

FROM THE AUTHORS' SUMMARY.

THE ELIMINATION OF THE BLOOD GROUP FACTOR IN SALIVA WITH GASTRIC JUICE. B. I. FOG-MOELLER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **84**:359, 1935.

Patients with achylia gastrica associated with pernicious anemia and with various other diseases and patients with hyperchylia eliminate the blood group specific factors in the same way as do normal persons.

I. DAVIDSOHN.

THE ISOLYSIN IN NEW-BORN CHILDREN. P. MARCUSSEN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **84**:420, 1935.

Using Thomsen's improved technic, Marcussen demonstrated a strong though low-titered isolysin in 25.6 per cent of new-born children. The lysin was also demonstrated in 95 per cent of the mothers, including every mother of a child in whom lysin was present. The tests were carried on without addition of complement because a fairly constant and ample amount of it was demonstrated in all new-born children.

I. DAVIDSOHN.

THE THERMAL RANGE OF TYPHOID IMMUNE SERUMS. L. HIRSZFELD and R. AMZEL, Ztschr. f. Immunitätsforsch. u. exper. Therap. **84**:455, 1935.

Agglutination of red blood cells is more pronounced at low temperatures, while agglutination of typhoid bacilli is favored by higher temperatures. The thermal range of the flagellar (H—) agglutinins of the typhoid immune serums is wider (from 0 to 55 C.) than is that of the somatic (O—) agglutinins (from 10 to 55 C.). The technic of the preparation of separate antigens with the two types of agglutinogens is given. Hirszfeld and Amzel recommend a simple technic for the Widal test which brings out both forms of agglutination. Live bacteria are used. The dilutions are placed in the icebox for twenty-four hours, when the coarse (H—) agglutination is recorded. That is followed by incubation in the water bath at 45 C., which brings out the fine (O—) agglutination.

I. DAVIDSOHN.

Tumors

CHORDOMA: A STUDY OF ONE HUNDRED AND FIFTY CASES. R. E. MABREY, Am. J. Cancer **25**:501, 1935.

Chordoma arises from remnants of the fetal notochord. It is found twice as often in the sacral as in the cranial region. It sometimes involves the vertebrae. It may occur at any age but is seen more often in the "cancer age group." It is twice as common in men as in women. There are no characteristic symptoms. The diagnosis rests on the presence of a tumor in the sacral region and a defect in the sacrum, together with a section showing large vacuolated cells and a homogeneous mucinous-like substance. The prognosis is not good. Treatment in the sacrococcygeal cases is surgical whenever possible. Treatment with the roentgen rays and radium is probably of some value in advanced cases.

FROM THE AUTHOR'S SUMMARY.

BONE METASTASIS IN CARCINOMA OF THE STOMACH. H. D. KERR and R. A. BERGER, Am. J. Cancer **25**:518, 1935.

Bone metastasis from carcinoma of the stomach is relatively uncommon. One hundred and forty-three reported instances have been collected, and five additional instances have been added to the series. Bone metastasis is most frequent at the sites of red marrow—the spine, ribs, femurs, sternum and pelvis. Metastases are either osteoplastic, osteoclastic or both, without regard to the characteristics of the primary lesions. The site, size and type of the primary tumor seem to have nothing to do with the appearance of osseous involvement. Bone metastasis is more frequent in relatively young persons, although it may occur at any age. Dissemination is probably through the blood stream. Some of the patients show an anemia which morphologically cannot be distinguished from the primary type and which may present a large increase in immature cells of the myeloid series.

FROM THE AUTHORS' SUMMARY.

MALIGNANT MYOMA. J MCFARLAND, Am. J. Cancer **25**:530, 1935.

The author concludes with Döring that the occurrence of metastasis is the only proof of malignancy in myoma.

CARCINOMA OF THE STOMACH IN IDENTICAL TWINS. R. E. MILITZER, Am. J. Cancer **25**:544, 1935.

Several points in the cases reported here are particularly noteworthy, namely, the marked resemblance of the brothers to each other, the almost simultaneous appearance of identical symptoms at the age of 70, the rapid progression of the disease, the symmetrical location of the tumors in the stomach and their similarity on pathologic examination. The age of these twins, 70 years, is considerably greater than the ages reported in similar cases. The tendency seems to be for such tumors to develop in childhood or early adult life.

FROM THE AUTHOR'S DISCUSSION.

PRIMARY MELANOSARCOMA OF THE LEPTOMENINGES. A. J. E. AKELAITIS, Am. J. Path. **11**:591, 1935.

Chromatophores normally present in the pia can take on melanoblastic functions. It is suggested that the chromatophore is a resting mesodermal melanoblast. Under pathologic conditions, for example, a malignant condition, the chromatophore can resume its melanoblastic activity and give rise to a primary melanosarcoma of the leptomeninges. This is, Akelaitis believes, what occurred in the case reported here. The development of typical stellate and spindle-shaped chromatophores from round

pigment cells is suggested by the pigmented flecks in the pia. The parenchymatous involvement was secondary and occurred by way of the perivascular spaces. The various types of pigmented cells seen in the tumor nodules of the parenchyma were identical with those found in the pigmented flecks of the pia.

FROM THE AUTHOR'S CONCLUSIONS.

THE PRODUCTION OF SARCOMA BY A SOLUTION OF 1:2:5:6-DI-BENZANTHRACENE. E. BOYLAND and H. BURROWS, J. Path. & Bact. **41**:231, 1935.

Repeated injections of an aqueous colloidal suspension of 1:2:5:6-di-benzanthracene produced sarcoma in rats and mice. The production of tumors did not appear to be affected by a change of diet or by an injection of silica remote from the site of injection of the hydrocarbon. The subcutaneous injection of a single dose of 1:2:5:6-di-benzanthracene up to 1 mg. did not produce tumors in rats. Repeated injections of the suspension into rabbits did not produce tumors. Repeated injections of colloidal solutions of anthracene, 1:2-benzanthracene and chrysene into rats also failed to produce tumors.

FROM THE AUTHORS' SUMMARY.

A FACTOR IN MALIGNANT TISSUES WHICH INCREASES THE PERMEABILITY OF THE DERMIS. E. BOYLAND and D. MCCLEAN, J. Path. & Bact. **41**:553, 1935.

Aqueous extracts of rapidly growing engrafted mammalian tumors contain a factor which, on intracutaneous injection into the rabbit, increases the permeability of the dermis. The factor is present in an amount which is approximately proportional to the rate of growth of the tumor. The more vigorously growing tumors yield much greater amounts of the diffusing factor than any normal tissue except mammalian testis, the extracts of which are active in high dilution. Extracts of other normal tissues tested in dilutions of 1:20 do not significantly increase the dermal permeability. Extracts of the rapidly growing fowl sarcoma 1 do not increase diffusion in the dermis. Extracts of the Fujinami myxosarcoma, on the other hand, produce an increased diffusion comparable with that caused by extracts of the more vigorous mammalian tumors. The inactivity of extracts prepared from the fowl sarcoma may be related to the poor yield of the diffusing factor obtainable from fowl testes. Extracts prepared from embryos and placentas of rats after different periods of gestation contain moderate but variable amounts of the diffusing factor.

FROM THE AUTHORS' SUMMARY.

THE HISTOLOGY OF PRIMARY CARCINOMA OF THE LUNG. K. LINDBERG, Arb. a. d. path. Inst. d. Univ. Helsingfors **8**:225, 1935.

In this elaborate monograph are reported the results of a systematic and thorough study of forty cases of pulmonary carcinoma. Special attention was given to the relations of the size of the nuclei to the nucleoli, and the results of extensive measurements are recorded. On the basis of cell morphology, the cases studied are divided into two groups: (1) those in which the cells were wholly or predominantly undifferentiated and (2) those in which the cells were mostly differentiated. The second group contains flat cell carcinoma, cylindric cell or adenocarcinoma and carcinoma of both these types. To classify a given case of pulmonary carcinoma on the basis of a study of one or a few sections, which often is done, no doubt frequently results in error. Lindberg could not establish any parallelism between the grade of atypical nuclei and the grade of malignancy. In each of the groups just mentioned there may occur alveoli with a distinct peripheral layer of elongated cells, arranged radially and in palisades, the nuclei of which stain more deeply than the nuclei of the other cells. It appears that these palisade cells may become transformed into more highly differentiated cylindric cells. In the carcinoma that consists mostly of apparently undifferentiated cells small adenomatous formations may be seen as well as small districts with hornifying flat

epithelium. It appears as if carcinoma with differentiation in two directions is not especially rare, but the discovery of its presence requires a thorough examination of the whole tumor.

EXPERIMENTAL PRODUCTION OF ATYPICAL MYOBLASTOMA OF THE TONGUE. B. MORPURGO, Centralbl. f. allg. Path. u. path. Anat. **63**:1, 1935.

After extensive studies of two myoblastomas of the tongue and one of the calf muscles Morpurgo concluded that the genesis of such tumors was not apparent in fully developed specimens. He attempted, therefore, to produce such tumors by injecting nicotine into the tongues and calf muscles of ten rats and succeeded in the tongue of one of the rats. This tumor was an atypical myoblastoma composed of small round cells rich in chromatin and longer cells with numerous granules in the cytoplasm. The picture seemed best explained on the basis of dysontogenesis rather than on that of hyperregeneration.

GEORGE RUKSTINAT.

CHANGES IN THE BLOOD PROTEINS IN MULTIPLE MYELOMA. W. GROS, Deutsches Arch. f. klin. Med. **177**:461, 1935.

Two cases of multiple myeloma are described in one of which there was a hyperproteinemia due to increase in pseudoglobulin. It is obvious that in myeloma there may be different disturbances in the protein metabolism, and the nature of these differences is not explainable by the structure of the cells. It is assumed that functional disturbances in cells in the marrow may be the underlying factor. The blood in myeloma may give a positive Takata reaction without apparent disturbances of the function of the liver.

PRODUCTION OF CANCER IN RABBITS DUE TO ADMINISTRATION OF TOBACCO TAR. F. H. LÜ, Frankfurt. Ztschr. f. Path. **46**:513, 1934.

Tobacco tar is an aqueous solution of the products of the distillation of tobacco at a temperature of between 400 and 500 C. Application of coal tar over a period of one hundred and twenty days may produce cancer in rabbits, whereas application of tobacco tar takes much longer. Tobacco tar, however, produces increased susceptibility to coal tar so that when the latter is applied cancer is more readily produced. The production of cancer by tobacco tar is also markedly accelerated by the application of coal tar to a different region of the body. This seems to confirm the observations of others (Beck, Fischer-Wasels) concerning the general effects of the application of coal tar as factors in the production of susceptibility to cancer. The sudden administration of a large dose of cholesterol after prolonged application of tobacco tar resulted in the production of cancer in a shorter time than observed by other writers. Lü believes that a sudden change in the proportion of cholesterol in the organism is a more important influence. Also treatment with cholesterol before the application of coal tar increases susceptibility to the development of coal tar carcinoma. However, if the treatment with cholesterol is given before the application of tobacco tar, it does not increase susceptibility to the development of tobacco tar carcinoma.

FROM THE AUTHOR'S SUMMARY.

Society Transactions

LOS ANGELES PATHOLOGICAL SOCIETY

CLARENCE M. HYLAND, *President*

Regular Meeting, Oct. 8, 1935

JOHN W. BUDD, *Secretary*

INTRA-OSSEOUS NERVE SHEATH SARCOMA OF THE FEMUR: REPORT OF A CASE. J. W. BUDD.

The summary of this article will appear in a later issue.

EARLY RENAL TUBERCULOSIS: REPORT OF A CASE. EMIL BOGEN.

Of 8,000 patients admitted to the Olive View Sanatorium, 250 have been specifically examined for evidences of renal tuberculosis. Of these, thirty had acid-fast bacilli in the urine as demonstrated by direct smear of centrifugated urinary sediment, and thirty-four others whose urinary sediment showed no bacilli on smear were shown to have renal tuberculosis by inoculation of the urine in guinea-pigs: a total of 64 cases in this series.

Twenty-two had nephrectomies elsewhere, and ten at Olive View Sanatorium. The kidneys removed have generally shown large tuberculous cavities with pelvic ulceration, and frequently there have been tuberculous nodules in the bladder on the side of the involved kidney. In a number of instances operation was not done because the renal disease was bilateral. Miliary tuberculosis was frequently found in the kidneys in patients who died of pulmonary tuberculosis, but in only a few patients has autopsy revealed tuberculous ulcerative lesions of the kidneys that had not been suspected during life.

Because delay in operation sometimes results in involvement of the other kidney and a hopeless prognosis, special efforts have been made to make the diagnosis of renal tuberculosis as early as possible. Accordingly, all urines showing pus cells or blood on initial examination are examined microscopically for acid-fast bacilli, and if these are not found the urine is inoculated in a guinea-pig.

The differentiation of tubercle bacilli from smegma bacilli or other nonpathogenic acid-fast bacilli is often not possible from microscopic examination alone, although organisms that decolorize with Pappenhem's stain are probably not tubercle bacilli. But the final decision is best based on inoculation of animals resulting in tubercle-bacilli from the glands, in a positive tuberculin test and in positive observations at autopsy.

Most urologists and investigators of tuberculosis seem to believe that if tubercle bacilli are found in the urine they represent tuberculosis in the genito-urinary tract, and that if the epididymis shows no signs of involvement the kidney is probably infected. Finding acid-fast organisms in ureteral catheterization is considered sufficient warrant for removing the kidney, and rarely is the surgeon disappointed in finding gross involvement of this organ. A few cases in which such involvement has not been found have been reported, it is true, but the instances were without adequate microscopic study of the kidney or inoculation of the ground-up renal tissue in animals.

The following is reported as an instance of early renal tuberculosis in which only a few organisms appeared in the urine and in which special search was needed to show the presence of a microscopic lesion.

A 34 year old man was readmitted in July 1934 with an irritation of the bladder and a recurrence of spinal pain. His previous history included treatment beginning in 1924 for active pulmonary and vertebral tuberculosis, with satisfactory results.

On cystoscopic examination in November 1934 urine was obtained from the left kidney and the bladder which gave positive results on inoculation into guinea-pigs. This examination was repeated with similar results in March 1935, and the left kidney was removed in July. Following this the symptoms pointing to involvement of the bladder disappeared.

The kidney removed was of normal size and appearance and showed no gross lesions on the surface or on several sections. Accordingly, it was sliced into thin pieces about 2 mm. thick, and the entire kidney was gone over grossly before being put in solution of formaldehyde, but no gross lesions were found. Every slice, therefore, was embedded in paraffin and sections stained. After considerable tedious examination of negative sections, the section presented here was found. Although several portions of the kidney showed collections of fibrous tissue or of lymphocytic infiltrate, this was the only lesion resembling a tubercle which was seen. It is possible, however, that others as small or smaller escaped observation, but these could not have been many or larger.

The lesion shows a central necrotic area, surrounded by lymphocytes and mononuclear or epithelioid cells, with no giant cells and little evidence of fibrosis. Attempts to locate the block from which it came, for the purpose of making acid-fast stains, were unsuccessful. It is held, however, that this is sufficiently typical in view of the positive results from inoculations of the urine into guinea-pigs to warrant a diagnosis of early solitary tubercle of the kidney, and that this or a similar lesion may account for the findings.

DYSENTERY CAUSED BY SHIGELLA DYSENTERIAE OF A SLOW LACTOSE-FERMENTING TYPE. JOHN F. KESSEL.

This report has been published (Kessel, J. F.; Blakely, Lee, and Cavell, Korine: Amebiasis and Bacillary Dysentery in the Los Angeles County Hospital, *Am. J. Trop. Med.* **16**:417, 1936).

HISTOLOGIC DEMONSTRATION OF UREMIA BY PRECIPITATION OF XANTHYDROL UREA IN TISSUE. ALBERT F. BROWN and ARAM A. KRAJIAN.

This article was published in full in the January 1936 issue of the ARCHIVES, p. 96.

V. L. ANDREWS, *President*

Regular Meeting, Jan. 14, 1936

JOHN W. BUDD, *Secretary*

BACTERIAL ENDAORTITIS WITHOUT VALVULAR LESIONS. J. L. MASON.

Two cases of bacterial endaortitis without involvement of the aortic valve are presented as of unusual interest because of their rarity and the lack of any discoverable recent literature on the lesion in question.

CASE 1.—A 50 year old Negro laborer entered the hospital in coma. He had for some time complained of nonradiating precordial pain and moderate dyspnea on exertion but had been able to work till the day of admission, when he had abdominal and back pains and rapidly became unconscious. There was left hemiplegia with absence of the deep and superficial reflexes bilaterally. It was considered probable that there had been a right cerebrovascular accident. Death occurred twelve hours after admission.

At autopsy, six hours later, a few conjunctival petechiae were observed. The heart was essentially normal; particularly was there no evidence of valvular defect or of vegetative formation on the endocardium. The aorta showed adjacent to the valve irregular intimal thickening with pearly elevations and some longitudinal wrinkling. In addition there were several large red vegetations that were rather loosely attached to the intima and friable. When these were removed, the underlying intima was found free from ulceration. Sections of these vegetations revealed typical thrombotic structure and numerous gram-positive cocci. On culture of the lesions Streptococcus viridans was recovered. Sections of aortic wall showed changes typical of syphilitic mesaortitis. Multiple emboli were discovered in the brain, kidneys and wall of the small bowel.

Case 2.—A 36 year old Caucasian was admitted to the hospital with facial erysipelas of one day's duration. One week later he was seized with diffuse abdominal cramps accompanied by nausea and vomiting. A diagnosis of acute appendicitis was made and a fairly normal appendix removed. Two days later the pain and distention were marked. The abdomen was silent. Death followed in a few hours.

Postmortem examination revealed a normal heart except for two small hemorrhagic areas in the endocardium of one papillary muscle. The aorta was notably smooth and free from atherosclerosis. Three centimeters above the aortic ring there was a brownish red vegetation which measured approximately 1 by 2 cm. This was ragged and rather loosely attached to the smooth underlying intima. Cultures from this showed a growth of Streptococcus viridans. Microscopic sections of the vegetation revealed findings similar to those described in the first case. The abdomen disclosed the changes characteristic of mesenteric thrombosis involving the small bowel and ascending portion of the colon. Careful dissection of the superior mesenteric artery showed near its origin an antemortem clot, which was considered an embolus. In addition, a recent large infarct was located in the right kidney.

Comment.—The observations in each case support the impression of an infectious process analogous to classic subacute bacterial endocarditis without the chronic valvular lesions on which the vegetative lesions are usually superimposed, the lesions being located solely on the aortic intima. In one of the two cases evidence of syphilitic mesaortitis was present but without evident involvement of the aortic valve.

Cardiac Rupture Associated with Metastases to the Heart from Carcinoma of the Duodenum. W. L. McNamara.

A white man of 48 years was admitted to the hospital in 1930 and remained there until he died four years later. His symptoms pointed to chronic valvular heart disease. In 1934 the heart was markedly decompensated. For seventeen days before death the patient suffered agonizing precordial pain and dyspnea. At times he suffered from epigastric pain and vomited blood.

At autopsy the pericardial sac was enormously distended with 500 cc. of blood. The heart was enlarged, weighing 700 Gm. The epicardium was covered with firmly adherent fibrinous-like reddish material. The left auricle measured 10 cm. in diameter, and on removal of the heart the auricular wall was torn, a large portion remaining attached to the parietal pericardium. The auricular wall was 8 mm. in thickness. The endocardial surface, including that of the mitral valve was almost completely covered with a verrucous-like growth. The point of rupture was found on the posterior wall in the portion that was adherent to the pericardium. The mitral valve showed a fish mouth type of deformity. The aortic valve showed evidence of some old inflammatory lesion, probably rheumatic.

On the posterior duodenal wall, close to the pyloric ring, was a small ulcer 5 mm. in diameter. The walls and base were indurated, and the edges were crateriform. The lesion showed active ulceration with some bleeding. The regional

lymph nodes were not enlarged. Microsection of the duodenal wall in the region of the ulcer showed a diffuse infiltrating type of new growth with cells of round, oval and polyhedral type, varying markedly in size. The nuclei were relatively large and somewhat hyperchromatic. For the most part the cells were growing in columns and cords and infiltrated the duodenum profusely. There was some attempt at alveolar formation, but for the most part the growth was quite anaplastic. Practically no desmoplasia was present.

The myocardium of the left auricle was infiltrated by epithelial cells having the same appearance as those noted in the tumor of the duodenum. The endocardium of the left auricle was covered with a layer of closely packed epithelial cells of the same appearance.

It is concluded that metastases to the heart from a primary carcinoma in the duodenum weakened the myocardium and precipitated cardiac rupture in a patient with rheumatic heart disease.

RHEUMATIC PNEUMONIA. L. J. TRAGERMAN.

A Mexican housewife aged 30 entered the hospital in the fourth month of pregnancy because of cough for one month and dyspnea, sore throat and blood-tinged sputum for twenty-four hours. On examination she was noticeably dyspneic and appeared acutely ill. The temperature was 97.2 F.; the pulse rate, 142, the respirations, 32. There was evidence of consolidation in the upper lobe of the right lung, and a soft systolic murmur was heard at the cardiac apex. The temperature varied between subnormal and 100 F., being for the most part subnormal or normal. The condition improved suddenly on the sixth day and remained so for five days, following which cyanosis and dyspnea returned, and she died within twelve hours.

At necropsy the essential changes were in the heart and lungs. The heart weighed 350 Gm. and showed chronic rheumatic mitral and aortic valvulitis with stenosis, as well as an acute verrucous aortic and tricuspid rheumatic endocarditis. The pericardium was normal. The right pleural cavity contained 200 cc. of clear fluid, and the pleural surfaces were smooth and glistening. The right lung weighed 1,025 Gm. The cut surfaces of all lobes were diffusely consolidated, brick red and rubbery. The vessels, bronchi and lymph nodes were normal. The left pleural cavity was dry, with loose fibrous adhesions laterally in both lobes. The cut surfaces were mostly crepitant, hyperemic, rather dry and pink brown, suggesting chronic passive hyperemia. In addition there were scattered irregular areas of consolidation, varying up to 3 cm. in maximum diameter, with an appearance similar to that of the entire right lung. Microscopic sections through these areas and through all the lobes of the right lung showed uniform changes. There was some thickening of the subendothelial layers of the pleura through the presence of loosely woven collagenous fibers containing connective tissue cells, lymphocytes, erythrocytes and endothelioid cells. All alveolar walls showed varying degrees of thickening. When slight, this was due simply to edema and capillary congestion. For the most part it was due to varying degrees of cellular proliferation or infiltration, often with formation of submiliary nodules which projected deep into the alveolar lumens. The predominant cells in this interstitial pneumonitis were the large endothelioid cells with darkly stained nuclei. Occasionally multiple paler elongated cells resembling epithelioid cells were seen. Also present were polymorphonuclear leukocytes and scattered fibroblastic proliferation. Small bronchi and bronchioles were invaded by a similar process. The blood vessels, many of which were surrounded by collars of this exudate, were themselves unaltered. The interlobar septums were thickened and infiltrated by endothelioid cells, but no Aschoff bodies were seen. Most of the alveoli contained the so-called heart failure cells and what appeared to be desquamated epithelium. Some were empty. With the Pappenheim methyl pyronine stain, the endothelioid cells as well as the cartilage cells and mucous glandular epithelium stained light red. Direct smears from the

lung showed no organisms. However, in one culture a growth of Pneumococcus was obtained which failed to react with any of the standard Neufeld serums.

The existence of specific pneumonitis in rheumatic fever has been disputed. The best morphologic evidence has been presented in the reports of Naish and of Eiman and Gouley. In this patient the clinical picture was that of pneumonia, and the patient died without other apparent cause, e. g., congestive heart failure. The most striking observation at necropsy was an unusual lobar consolidation of the right lung. Histologically it proved to be due to interstitial pneumonitis, which was characterized by a cellular reaction often seen in rheumatic disease.

Coccidioidal Granuloma: Pulmonary Lesion of Four and a Half Years' Duration. H. A. Ball.

A man of 66 years had "pneumonia" in December 1930. One month later he noted a subcutaneous swelling on the forehead. This gradually increased in size for four months and then was incised. Tissue was removed and the diagnosis of coccidioidal granuloma established. One month later the frontal bone beneath the lesion was curetted because of osteomyelitis. Shortly thereafter x-ray pictures of the chest showed right basilar infiltration extending downward from the hilar area. Cultures from the lesion on the forehead were positive for the fungus Coccidioides. Wet dressings of 5 per cent potassium iodide solution saturated with iodine (3.9 per cent) were applied, and healing of the lesion was effected after six months of treatment. The response of the local lesion was reported in *The Journal of the American Medical Association* (**98**:2279, 1932). Since then a case has been reported by Sorsky and Nixon, who obtained similar results with the use of this solution (*California & West. Med.* **42**:98, 1935). The lesion did not recur in the case under discussion, nor was there any evidence of a similar process elsewhere.

In June 1935 this patient was readmitted to the hospital with hematuria, albuminuria, nitrogen retention and uremia, from which he died. There was no evidence of an active infectious process during this terminal illness.

At autopsy the depressed scar in the forehead showed no active inflammation. The external scar was continuous with a dural scar through a defect in the frontal bone 2 cm. in diameter. There were no adhesions of the leptomeninges at this point. The lungs were partially adherent in their pleural cavities. The right lung presented at the base an elongated area of fibrous and chalky caseation measuring 2 by 3 by 6 cm. The kidneys were of normal size but hemorrhagic, without evidence of chronic scarring.

Microscopic studies of the pulmonary lesion showed extensive fibrosis arranged concentrically about caseous foci. Within the caseous material and particularly within the proximal zone of connective tissue literally scores of hyaline bodies of the fungus Coccidioides were seen imprisoned. No inflammatory exudate surrounded many of the organisms. They appeared embedded in old connective tissue. Some of the models showed chronic inflammation surrounding the fibrous tissue, but most did not. Extensive search failed to reveal a single sporulating organism, indicating that reproduction was at a standstill. Some of the capsules were collapsed and partially destroyed, but most of the visible forms were certainly viable organisms. The kidneys showed glomerulonephritis.

Comment.—The pulmonary lesion corresponded in size and position to the infiltration demonstrated by x-ray picture six months after the disease started. Since in practically all cases of this disease the atrium of infection is the lungs, it is reasonable to assume that the pulmonary lesion existed from the time of the so-called pneumonia. This indicates that the fungus Coccidioides had been present in the lung for four and one-half years when the patient died. Since on microscopic examination no reproducing forms could be detected we are forced to assume that either reproduction was cyclic and we observed the lesion during the resting stage or that there was an unexpected longevity of the organism in the presence of obvious defense efforts on the part of the body.

Since it is certain that during the initial dissemination of the disease the organisms gain entrance to the blood stream and become deposited in the peripheral tissues, it seems clear in this case as in many others that this process was never repeated. Observations in numerous cases indicate that the lung is better able to take care of itself in the presence of this infection than most other types of tissue, so that if a second dissemination occurs it very likely produces observable lesions. Since such lesions did not occur in this case it seems probable that the scarring effected complete protection against subsequent entrance of the organisms into the circulation. The observations thus explain what probably happens within the lung in most cases. Since in some cases new peripheral lesions have been known to develop two years or more after the first, it follows that the result of pulmonary fibrosis is not always so effective as in the case presented.

BUFFALO PATHOLOGICAL SOCIETY

Regular Meeting, Nov. 27, 1935

KORNEL TERPLAN, *President*

WILLIAM F. JACOBS, *Secretary*

ACUTE MESENTERIC VENOUS THROMBOSIS FOLLOWING THROMBO-EMBOLISM OF THE SPLENIC ARTERY WITH INFARCTION OF THE SPLEEN. ELMER MILCH.

The following case of mesenteric venous occlusion is reported because of the comparatively rare sequence of etiologic factors concerned in its production.

A white man 53 years of age was admitted to the hospital with a history of dull gnawing pain in the left hypochondrium of four weeks' duration. Just before entry the pain became excruciating, and he collapsed. Frank hematemesis and bloody diarrhea followed. The patient was in severe shock. The temperature was 100 F. by rectum; the pulse rate, 140; the respirations, 12. The blood pressure was 104 systolic and 86 diastolic. The abdomen showed only marked tenderness in the epigastrium. The white cell count of the blood was 35,000, with 93 per cent neutrophils. The patient failed rapidly and died twenty-six hours after admission. Necropsy was done thirty minutes after death.

The pertinent anatomic abnormalities were: total and partial occlusion of the circumflexing and oblique descending branches of the coronary arteries; extensive fibrous myomalacia of the left ventricle; polypous vegetations attached to the endocardium; recently localized parietal thrombi at the lower portion of the thoracic aorta; complete occlusion of the distal portion of the splenic artery by an old thrombo-embolus; multiple infarctions of the spleen; obliteration of the distal portion of the splenic vein by an organized thrombus arising in an infarcted area of the spleen; more recent ascending thrombosis in the proximal portion of the splenic vein; entirely recent massive thrombosis of the mesenteric vein and portal vein, with recent hemorrhagic infarction and necrosis of the upper jejunum; about 1,000 cc. of blood-tinged fluid in the peritoneal cavity.

The terminal venous thrombosis is regarded as a direct extension of the older thrombosis of the splenic vein, which in turn originated in an embolic infarction near the hilus of the spleen. The source of the embolus was most likely parietal thrombi in the heart.

It has not been possible to find in the literature any record of a case that was similar to the one reported here.

RUPTURE OF THE ANTERIOR PAPILLARY MUSCLE OF THE LEFT VENTRICLE. WILLARD H. CLEVELAND.

This is the twenty-first recorded case of rupture of a papillary muscle in the heart. It occurred in a 55 year old colored laborer, who complained of precordial pain and dyspnea of one year's duration. The heart was enlarged in all diameters. A soft systolic bruit was heard at the apex and was transmitted to the left axilla. The cardiac rate was regular and the tone good. The blood pressure measured 230 systolic and 175 diastolic. Bilateral pulmonary congestion with hydrothorax, ascites and an enlargement of the liver were present. The Wassermann reaction of the blood was 4 plus. Under rest and treatment the patient became ambulatory, and a pulse rate of from 80 to 90 was maintained. Then he disregarded the specific caution not to climb stairs and visited the floor above three times. Soon afterward he suffered agonizing precordial pain, became markedly dyspneic, and expired sixty-four hours later.

Autopsy revealed a massively enlarged heart, weighing 660 Gm. The wall of the right ventricle measured 0.4 cm.; that of the left, 2 cm. The coronary vessels showed sclerotic thickening without thrombosis. The chordae tendineae of the anterior mitral leaflet were separated from the anterior papillary muscle. The tip of the anterior papillary muscle showed hemorrhagic necrosis. A fragment of the extreme tip had been torn off and was still attached to the chordae tendineae. The remainder of the papillary muscle was grossly normal.

A study of serial sections through the papillary muscle and the myocardial wall at its origin revealed sclerosis of the arteries from base to tip, with occlusion by fibrous thickening of the intima. The surrounding muscle fibers revealed patchy alternation of scarring, hemorrhage and acute degenerative changes.

It is concluded that the slowly progressive coronary disease was responsible for the atrophic disappearance and fibrous replacement of the muscle elements in this papillary muscle, with the ultimate development of an area through the tip that could not withstand the strain of sudden additional effort.

In accounting for this rupture two factors seem to be responsible; one the upward tension against the undersurface of the mitral leaflet by force of the blood during ventricular systole (Voit), and the other the contractile force of the papillary muscle. Because of these opposing forces the rupture was brought about at the point of greatest involvement.

CONGENITAL TUBERCULOSIS IN A SIX WEEK OLD INFANT. K. L. TERPLAN and O. HOSTERMAN.

A 6 week old infant was admitted to the Children's Hospital because of irritability and failure to gain weight since birth. No abnormalities of the placenta had been noticed at the delivery, which took place in a private home. Two months previous to confinement the mother suffered from a "cold" during which she raised much sputum. Following delivery this "cold" recurred. For two weeks after birth the infant was breast fed. On admission the infant showed marked jaundice. The temperature was 101 F.; the liver and spleen were easily palpable. The laboratory findings included 47 per cent hemoglobin, 2,800,000 red blood cells and 4,400 white cells per cubic millimeter, with polymorphonuclears 70 per cent, lymphocytes 19, mononuclears 2 and nucleated red cells 11 per cent, and blood sugar, 20 mg. per hundred cubic centimeters. The jaundice increased; the breathing became more and more labored. Roentgen examination showed scattered small areas of pneumonic consolidation in the right lung. The infant died apparently from pneumococcic meningitis following purulent otitis media. A culture from the spinal fluid revealed Pneumococcus. Postmortem examination of the brain was unfortunately restricted.

Further investigation of the family revealed that the father, five brothers and one sister were free from tuberculosis. The mother, however, showed by roentgenogram chronic pulmonary tuberculosis, especially at the base of the left lung. Her

spine displayed evidence of Pott's disease at the fourth dorsal vertebra, a disease from which she first suffered eight years previously and for which she had a cast applied to her back for forty-six weeks.

The postmortem observations were as follows: extensive caseation with beginning cavity formation in the periportal lymph nodes, which measured 2 by 1.1 cm.; complete caseation of the lymph node at the neck of the gallbladder—the node was about the size of a bean; thousands of cheesy tubercles in the liver, varying in size between a submiliary nodule and a small pea-sized one; an enlarged spleen with diffuse small miliary tubercles; extensive caseation of the periaortic lymph nodes, which measured about 1 by 0.5 cm.; scattered cheesy tubercles in all lobes of both lungs, varying in size from that of a pinhead to that of a pea; a few cheesy tubercles measuring less than 2 mm. in diameter in the periphery of both lower and upper tracheobronchial lymph nodes; a few scattered miliary tubercles in the kidneys and in both adrenal glands; a few recent tubercles in the mucosa of the small intestine and a few conglomerate pinhead-sized to pea-sized cheesy tubercles in several mesenteric lymph nodes, which, however, were not enlarged.

The anatomic picture points, in our opinion, to congenital tuberculosis. The primary lesions were apparently those scattered over the liver. Marked caseation of the periportal lymph nodes with beginning cavity formation and the absence of similar marked changes in the tracheobronchial lymph nodes point to the liver as the probable portal of entry. In line with this is the extensive caseation found in the periaortic lymph nodes. The distribution and size of the tubercles in the lungs point to a hematogenous rather than to a bronchogenic origin. In line with this are the relatively minor tuberculous lesions in the tracheobronchial lymph nodes and the entire absence of a tuberculous complex such as one usually sees in primary aerogenic tuberculosis of the lungs. Although no large primary focus of infection has been found in the liver, all the findings together point to a primary hematogenous congenital tuberculosis. The umbilical vein was obliterated, and the cord did not show any tuberculous lesions. In spite of the fact that the placenta had not been examined and that there was no clinical evidence of tuberculous endometritis in the mother, we can hardly explain the anatomic findings in any way other than by placentogenic miliary tuberculosis. In the anatomic type and distribution of the tuberculous lesions our case resembles somewhat that published by Rollett in 1909.

MASSIVE BRONCHOGENIC PULMONARY TUBERCULOSIS IN A THREE WEEK OLD INFANT. FRANCIS J. GUSTINA and WILLARD G. CLEVELAND.

A 20 day old white infant, a girl, was admitted to the Buffalo City Hospital Sept. 20, 1935. Her delivery was by an uneventful vertex presentation. The ninth day after birth she began to have attacks of cyanosis and frequent panting respirations with some cough. The temperature ranged between 100.5 and 101.8 F. Roentgen examination of the thorax the day of admission showed a diffuse fine mottling throughout both lungs, and a diagnosis of miliary tuberculosis was made. The child's condition became progressively critical. The attacks of cyanosis, apnea and hyperpnea became more frequent. She had taken her feedings well for over two weeks but then began to regurgitate, and September 24 she died. The Mantoux test with 0.1 cc. of tuberculin, done forty-eight hours before the child died, was negative. The findings in the spinal fluid were negative.

The postmortem observations were: diffuse massive bronchogenic tuberculosis with caseation of the peribronchial lymph nodes, more marked at the left hilus; a well circumscribed hemorrhagically congested area in the lower lobe of the left lung with the overlying pleura slightly elevated and with distinct caseating pneumonia in the immediate surrounding tissue. No cavity formation was present. Histologic sections showed typical exudative tuberculosis. By the Ziehl-Neelsen method, myriads of acid-fast bacilli were found.

The umbilical cord, gastro-intestinal tract, upper respiratory tract, pharynx, oral cavity, spleen and liver were entirely normal.

It was learned that seven years previous to confinement the mother had suffered from pleurisy with effusions. However, at no time were tubercle bacilli found. At delivery of this child no signs of active tuberculosis were found in the mother, nor was there any evidence of tuberculous endometritis. Several weeks after the postmortem examination of the child the mother was carefully reexamined. X-ray pictures of the lungs and spine, physical examination and bacterioscopic examinations of sputum all failed to disclose tuberculosis.

Inasmuch as no contact with tuberculous infection at and after the delivery of the child could be found, we refrain from deciding whether we are dealing with a prenatal bronchogenic tuberculosis following aspiration of tubercle bacilli from the amniotic fluid or with a primary aerogenic tuberculosis of the lungs in very early postnatal life.

DISCUSSION

K. TERPLAN: I am more inclined to consider this case as one of extra-uterine massive infection shortly after birth. The very early age of the infant does not necessarily indicate that this massive tuberculosis of the lungs may have started prenatally. The patients of Hauser and Zarfl both died at the age of 3 weeks. These conditions are recognized in the literature as not congenital but postnatally acquired tuberculous infections.

Regular Meeting, Feb. 29, 1936

KORNEL TERPLAN, *President*

WILLIAM F. JACOBS, *Secretary*

HYDROGEN ION PRECIPITATION OF WATER-SOLUBLE TUBERCULOPROTEIN. E. B. HANAN and W. P. ERICKS.

Various reagents were tried in our attempt to precipitate the water-soluble tuberculoprotein from solutions containing other organic substances. These were abandoned in favor of hydrogen ion concentration.

By the use of various buffer solutions it was established that the optimum p_H for precipitation was 2.8. This was confirmed and more thoroughly studied by employing the standard buffer solutions of Clark and Lub with a p_H range from 2.2 to 7.

The heat-extracted tuberculoprotein gave maximum precipitation first at p_H 2.8, but on standing over night the maximum range extended toward the alkaline side, to approximately p_H 4.6.

The unheated tuberculoprotein showed a more sharply limited range. The optimum precipitation occurred at p_H 2.8, but on standing over night the maximum range tended to extend toward the acid side.

Complement fixation and skin tests indicate that the tuberculoprotein precipitated at p_H 2.8 retains its antigenic properties.

It is suggested that further studies of the hydrogen ion precipitation method may be a distinct aid in the isolation and purification of tuberculoprotein. The low p_H precipitation would aid in separating the tuberculoproteins of albuminous nature.

A RECONSTRUCTION MODEL OF A SMALL PEPTIC ULCER. W. F. JACOBS.

A small ulcer of the stomach, about 2 mm. in diameter, found at autopsy in a patient 56 years of age who died of massive cerebral hemorrhage, was embedded. Serial sections were cut, and every fifth section was mounted. From these a reconstruction model was made which enlarged the ulcer twenty-five times. It was found that the vessel takes a definitely oblique course in the submucosa, as claimed by Frey and Mall.

MULTIPLE CYSTIC MALFORMATIONS SOMEWHAT RESEMBLING THE HIPPEL-LINDAU SYNDROME. WALTER PUTSCHAR.

Lindau has shown that the coexistence of angiomatous cysts in the cerebellum with cystic or tumor-like tissue malformations within some of the abdominal organs, especially the pancreas, is rather frequent. The latter sometimes were found only by microscopic examination. The question came up as to whether obvious cystic malformations of abdominal organs may not be combined with minute tissue malformations within the central nervous system of the retina.

The following case illustrates the problem and should stimulate interest in this type of combined tissue malformations. A white man 30 years of age died of uremia. Autopsy revealed large congenitally cystic kidneys, cystic dilatation of the seminal vesicles and of the ampullae of the deferent ducts, many small and large cysts in both epididymitides, diffuse ectasia of the rete testis, a few small cysts in the liver and along the splenic vessels within the fat tissue and finally a single subcapsular cyst in the spleen. The pancreas did not show macroscopic changes. Dissection of the brain revealed a tumor-like hempseed-sized whitish nodule in the left area vasculosa in the floor of the fourth ventricle.

Both eyes appeared macroscopically normal.

Histologic examination confirmed the diagnosis of congenital cysts in the kidneys, liver, spleen and epididymitides. The cysts along the splenic vessels were ectatic lymph vessels. The pancreas showed microscopically a few very minute cysts and peculiar band-shaped adenomatous formations resembling closely the structure of islets of Langerhans except for their more diffuse arrangement in the exocrine parenchyma. The head of the pancreas revealed an adenoma-like area formed by large ducts with papillary arrangement of their epithelium.

The nodule on the floor of the fourth ventricle consisted of glia tissue, rich in fibers but without signs of blastoma-like proliferations. Serial sections through both optic disks and the adjacent parts of the retina showed in each eye close to the margin of the disk a small lentil-shaped area made up of glia tissue and a few myelinated nerve fibers.

It is believed that these structures in the brain stem and in the eye are congenital malformations of the glia. It is of special interest that in Lindau's syndrome just the area vasculosa in the floor of the fourth ventricle is often the site of angioma, which is frequently combined with retinal angioma. In this case there was no angiomatous formation; however, the identity of localization of the glia tissue malformations is remarkable. The findings reported bring to mind a case published by Ledebur and Berblinger in which a glioma in the cervical part of the cord was combined with a cystadenoma of the pancreas.

LARGE SUPRAHYOID ACCESSORY THYROID. RAYMOND S. ROSEDALE.

In a colored woman aged 69 a convex swelling the size of a large plum bulged the undersurface of the anterior part of the tongue forward, pushing the dorsum upward and backward. This had been observed by physicians for about three years. The lateral lobes of the thyroid gland were palpable. The tumor beneath the tongue was removed easily. It measured 5 by 3.7 by 4.3 cm. It was ovoid and possessed a thin fibrous capsule. On the cut surface fibrous septums arose from the capsule to course into the tumor proper, which was fleshy in consistency, had glistening points and varied in color from a dead white to a pale pink yellow. A cyst in the tumor, containing a blood clot, and measured 3.5 by 2.5 by 1 cm.; the blood clot was adherent to its smooth shining wall. Other pinhead-sized points of hemorrhage were present in the fleshy portions.

Microscopically the typical picture of a parenchymatous struma with little colloid and an edematous hemorrhagic stroma with large thin-walled sinusoids was present. Mucicarmine stains were negative. The surgical removal of the growth was not followed by symptoms of hypothyroidism.

NEW YORK PATHOLOGICAL SOCIETY AND NEW YORK ACADEMY OF MEDICINE, SECTION OF PEDIATRICS

Combined Meeting, March 26, 1936

N. CHANDLER FOOT, *President, New York Pathological Society*

BÉLA SCHICK, *Chairman, New York Academy of Medicine, Section of Pediatrics*

MILTON HELPERN, *Secretary, New York Pathological Society*

ALEXANDER T. MARTIN, *Secretary, New York Academy of Medicine, Section of Pediatrics*

SPLENOMEGALY IN CHILDHOOD: PATHOLOGIC ASPECTS. DR. PAUL KLEMPERER.

The chronic splenomegalies in childhood can be classified according to morphologic-pathogenetic principles into six groups: (1) inflammatory, (2) infiltrative, (3) hyperplastic, (4) tumors, (5) cysts and (6) circulatory.

Each group has its characteristic histologic criteria. The various splenomegalies so far classified according to clinical principles only, such as splenomegaly in association with cirrhosis, Cooley's anemia or hemolytic anemia, can be included in this classification. The spleen in so-called Banti's disease and in splenic anemia shows no characteristic histologic features. The criteria postulated by Banti as characteristic for his disease can be found in a variety of splenomegalies of various pathogeneses. Continued histologic analysis in cases of conditions clinically suggestive of Banti's disease or splenic anemia will finally clarify the issue and lead to abandoning this diagnosis of convenience.

SPLENOMEGALY IN CHILDHOOD: CLINICAL ASPECTS. DR. RUSTIN MCINTOSH.

The clinician is under a handicap from the start because of his occasional uncertainty whether a mass occupying the left upper quadrant of the abdomen is the spleen. In cases in which the degree of splenomegaly is slight or moderate the organ tends to retain its normal shape and position, so that errors in diagnosis are unlikely, but in cases in which the enlargement is great, measures additional to those of routine physical examination must be applied. The most helpful of these in actual experience are excretion urography and fluoroscopy with the use of the barium sulfate enema.

It is well known that in children the spleen is more frequently felt on routine examination than is the case in adults. Among 151 unselected autopsies, the spleen was clinically palpable in 25 per cent. The spleens not felt during life on routine examination were in most cases of approximately average size for the patient's age but ranged as high as three times the average weight. Among the group of palpable spleens, a considerable proportion were of average size for the patient's age. The largest spleens were those of patients with Gaucher's disease, sickle cell anemia, kala-azar, congenital obliteration of the bile ducts, lymphoid leukemia, congenital hemolytic jaundice, erythroblastosis and congenital syphilis. Palpability of the spleen without conspicuous enlargement must not be assigned too much weight in differential diagnosis in young patients. Correspondingly, no particular advantage is to be expected from technical tricks which increase the proportion of spleens felt.

Classifications of causes of splenomegaly built up on a histopathologic basis are generally not satisfactory for clinical use. A grouping according to age incidence of the commoner causes of splenic enlargement has proved unsatisfactory except as applied to the neonatal period. Splenomegaly in the first month of life is usually related to syphilis, sepsis, congenital malformation of the bile ducts or erythroblastosis; beyond this period, however, the possibilities spread out rapidly.

A classification based on functional behavior of the spleen, while ideal in conception, fails utterly in practical work. Approximately seven distinct functions of the spleen have been verified in clinical investigations in human patients. In any determination of the causes of splenomegaly one's judgment on the basis of functional tests becomes obscured, because function does not invariably alter with a change in size, not all functions are simultaneously affected in the same proportion or direction, failing function of the spleen may be entirely compensated in some other part of the body and, finally, because not one of the tests gives results which can be interpreted as the product of changes in the spleen alone.

A clinical classification of the causes of splenomegaly is attempted under the following major subdivisions: (1) congenital and familial diseases, (2) mechanical factors, (3) infections, (4) neoplastic causes, (5) constitutional and nutritional factors, (6) splenomegaly in relation to other blood diseases not previously included and (7) splenomegaly of undetermined origin. On such a framework any clinician can build a serviceable diagnostic instrument.

In actual diagnosis little can be done about the spleen itself except to determine its size. Splenic puncture, which is staunchly advocated in many clinics, has relatively few advocates in New York. In splenomegaly one places special emphasis on studies of the blood. In addition to the usual clinicopathologic measures of counting and staining the cellular elements, one may resort to study of the bone marrow by biopsy or puncture and by the roentgen rays; also, the rapidity of destruction of erythrocytes may be inferred from the output of urobilin in the stools and the urine or, less satisfactorily, from the bilirubin level in the serum. In cases of splenomegaly with associated lymphadenopathy, biopsy of a node may reveal the presence of some factor operating in portions of the lymphoid or reticulo-endothelial apparatus outside the spleen and may permit the inference that analogous changes are present in these tissues within the spleen. In suitable cases intensive studies must be directed at the liver. In fact, any promising lead must be followed, and the diagnostic problem extends into all the fields of clinical medicine.

From the therapeutic point of view the enlarged spleen affords limited avenues of direct attack; it may be observed, irradiated or removed. As a matter of fact, it is in assessing the indications for splenectomy that the functional tests now available render their greatest service to clinical medicine. Aware of my temerity in trespassing on the surgeon's domain, I have discussed these indications briefly.

DISCUSSION

DR. MARGIT FREUND: The speakers of the evening have covered the field so completely that it will be difficult to say anything without repeating their statements. However, some of the points may bear repetition.

The clinical diagnosis of splenomegaly is simple in many instances and exceedingly difficult or almost impossible in others. With the aid of detailed hematologic investigations the group of leukemias, constitutional hemolytic jaundice, Cooley's anemia and even Niemann-Pick's disease can generally be identified. The diagnosis may be aided and completed by roentgen examination of the skeleton, as in Cooley's anemia and Gaucher's disease. If such investigative methods are not of help, puncture of the bone marrow may clarify the condition, as in instances of aleukemic leukemia or Gaucher's disease. In a case of kala-azar my associates and I found the Leishman-Donovan bodies. We have carried out this procedure in many cases within the last year and never had any accident. It is far less dangerous than splenic puncture.

With these methods of investigation the condition will be clarified in a considerable number of cases. However, in at least an equal number of cases, these laboratory methods are of no avail. These are cases of the condition which should be called cryptogenetic splenomegaly, in which, however, often a diagnosis of convenience, Banti's disease or splenic anemia, is adopted. The speakers of the evening have emphasized how unsatisfactory these diagnoses are. We have avoided their

use and have to try to come to a conclusion by using clinical considerations in cases in which laboratory methods fail.

A classic example of how the ingenuity of the clinical mind can solve such problems is the first case of thrombophlebitic splenomegaly seen by Türk in 1901. The patient was a boy of 10 who had splenomegaly and suffered from frequent hematemesis; the prevailing diagnosis was gastric ulcer, which left the splenomegaly unexplained. Türk noticed the cicatricial condition of the umbilicus and found out that the child had had a severe umbilical infection three months after birth. He correctly concluded that this naval infection had propagated into the umbilical vein and caused occlusion of the portal vein. In every case of unexplained splenomegaly with hemorrhages from the alimentary tract one must think of occlusion of the portal or the splenic vein. Wagner and Noble have compiled twenty-six cases dating up to 1933. My associates and I diagnosed such a condition, which was proved at autopsy by Dr. Lederer at the Brooklyn Jewish Hospital, and observed another case in which the histologic picture of the spleen spoke in favor of the diagnosis. The child is well one and a half years after operation.

The differential diagnosis is extremely difficult to make in cases of cirrhosis of the liver, which is not rare in childhood. Slight icterus, which is not rare in association with the latter condition preceding the splenomegaly might help in making the diagnosis. But with all the methods of laboratory and bedside investigation, splenectomy may still bring a surprise.

This occurred in the case of a girl of 14 who was referred to by Dr. Klemperer as showing hyperplastic splenitis. The patient was under the observation of my associates and me for six years. Splenomegaly had been noticed when she was 7½ years of age, a few months before she was admitted to the dispensary of the Mount Sinai Hospital. At the first examination of the blood the hemoglobin content was 70 per cent, and the white cell count was 8,000. Gradually there developed moderate anemia, a hemoglobin content of 58 per cent and leukopenia, the white cell count being 3,400, parallel to the striking increase in the size of the spleen. This case illustrates well the fact which Dr. McIntosh pointed out that leukopenia is not characteristic of any particular form of splenomegaly but is the result of depression of bone marrow by the large spleen. Nosebleed was frequent; once the child had hematemesis. She was much retarded in her development. She never had fever or any other evidence of a chronic infection. The parents refused permission for bone marrow puncture, and therefore Gaucher's disease could not be ruled out. The preoperative diagnosis was Gaucher's disease or thrombosis of the splenic vein. The report of the pathologic laboratory was hyperplastic splenitis. The child has been well since the operation in November 1934. Her hemoglobin content is 80 per cent, and the white cell count is 12,500. She has matured remarkably and has gained 30 pounds (13.6 Kg.). Such a case shows how far physicians still are from the goal of an exact diagnosis in splenomegaly in childhood.

DR. DAVID PERLA: A great deal has been said tonight about splenomegaly. I think that there is nothing more to say about it, but it might be interesting to pause for a moment and ask ourselves why we have a spleen. Is the spleen only for unusual circumstances, and is it interesting only in rare diseases? I have listed a number of functions about which I should like to say a few words. One must not forget that the spleen represents the largest collection of macrophage tissue in the body; that all the elements of the spleen arise from mesenchymal reticulum, the reticular cells, and that these cells have the capacity, under unusual circumstances and in normal development, of differentiating into a large number of elements. They may form free reticular cells, histiocytes or macrophages, or they may form lymphoblastic tissue and lymphocytes, or hemocytoblasts, and may give rise to the hematopoietic tissue and erythroblastic tissue or even to myeloblastic tissue. The mesenchymal tissue is a precursor of all the macrophage tissue and all the hematopoietic tissue in the body. Another fact to remember is that the spleen is an organ of great sinusoidal capacity and therefore is capable of

tremendous variations in size on account of its capacity to store blood. If these facts are kept in mind some of my remarks will be clearer.

The spleen serves the purpose of hematopoietic tissue in animals that have no bone marrow, such as the salamander, in which it is the source of production of red cells and thrombocytes. In embryonal life in human beings and in the higher mammals and under unusual circumstances during adult life or in childhood one finds evidence of hematopoiesis in the spleen; again, after a severe injury to the bone marrow, with the consequent necessity for this type of differentiation of this reticular tissue in the spleen, one notes its ability to form hematopoietic tissue. Dr. Klemperer brought that out clearly in an article in the "Libman Anniversary Volume"—this tremendous potentiality of the mesenchymal reticular cells for differentiation into the various elements at any time in life.

The question of the function of the spleen from the point of view of the blood reserve I think is relatively unimportant. Barcroft has laid a great deal of weight on that function, demonstrating the enlargement of the spleen following hemorrhage. There are many other organs which have the capacity of expansibility, and I think that this capacity to store blood under unusual circumstances is an unimportant function of the spleen. It merely means that the spleen has a tremendous capacity for variation in size. It alters in size many times during a lifetime; this is due to the elasticity of its tissue, which is variable in different animals, and the tremendous expansibility of this tissue.

There is also the question of bile pigment—whether it is formed in the reticular tissue in the spleen. But I think that by far the most important function of the spleen is its relation to resistance to infections. There is a tremendous amount of evidence from pathologic studies that the spleen is very important in combating acute and chronic infections. This evidence is inferential; there are anatomic changes in the spleen which indicate a tremendous stimulation of macrophage tissue, particularly in such diseases as malaria and trypanosomal and other protozoan infections. However, there is also considerable experimental evidence that the spleen is related to resistance. Removal of the spleen is followed in many animals by a drop in resistance to acute and chronic infections. In mice and rats removal of the spleen is followed by a marked lowering of resistance to tuberculosis, to infections due to Bacterium enteritidis, to spirochetal infections and to trypanosomal infections. It is interesting, however, that removal of the spleen in these animals is not associated with a marked drop in resistance to poisons, such as histamine; that is, the specific relationship of the spleen is evident only in certain types of infection. Even more interesting, I think, is the fact that removal of the spleen is followed in many species of animals by a flaring up of a latent infection. In mice, rats, dogs and other animals there are certain latent infections, such as infections due to Bartonella. Removal of the spleen converts these latent infections into manifest disease, and associated with this is the occurrence of severe infectious anemia. This is true of Bartonella anemia in the rat and mouse and of a great many diseases in the horse, the sheep and other animals. Such diseases are anaplasmosis in the sheep and Texas fever in cattle. The finding that the balance in the body between latent infection and manifest disease is controlled to a great extent by the presence of the spleen necessitates reconsideration of a great many facts which have been accumulated concerning the present knowledge of the physiology of this organ. The relation of the spleen to the iron metabolism is based on experimental work of this nature from which it was concluded that removal of the spleen is followed in certain animals by anemia. The interpretation was that removal of the spleen had resulted in some impairment of the utilization of the iron and of the formation of hemoglobin, and consequently the spleen was thought to play some part in the iron metabolism and the formation of hemoglobin, but it is known that this explanation is not correct.

Of late my associates and I have reported studies on the iron metabolism and the copper metabolism in certain strains of rats which were free from all latent infections. We found no relation of the spleen to the iron metabolism. There is

no change in the metabolism of iron in splenectomized animals, but there is a definite alteration in the metabolism of copper; that is, with removal of the spleen there is a tremendous outpouring of copper, with the establishment of a negative copper balance which persists for a considerable time.

Another point is the compensatory mechanism which follows removal of the spleen; this has a great clinical bearing on splenectomy. When one removes the spleen one stimulates all the hemoblastic tissue in the body. One can see that in any of a number of animals. There is a tremendous stimulation of the hemocytoblasts of the bone marrow, and there is the formation of ectopic foci of lymphocytic formation in the liver and lungs, so much so that the picture of the liver in a splenectomized rat six months after splenectomy closely resembles that in lymphoid leukemia. Clinical and experimental evidence has shown that there are a marked temporary increase in the number of blood platelets and a marked rise in the number of leukocytes for some time following removal of the spleen, so that all the derivatives of reticular tissue are stimulated by removal of the spleen. Once the compensatory mechanism has been established and all the other elements have been stimulated, the rest of the macrophage system takes up the functions of the spleen. The absence of the spleen is no longer detrimental, and after a certain period its removal is followed by sufficient compensatory hyperplasia of the other elements, which take over its function as regards natural and acquired resistance. After a certain time an animal subjected to splenectomy at an earlier date can no longer be infected with the manifest diseases to which the animal that has just been splenectomized is subject.

Finally, I should like to say a word about the relation of the spleen to central control. In the last year my associates and I have studied the relation of the spleen to the hypophysis, and in a week or so a paper will be published which shows that there is a definite factor in the anterior lobe of the pituitary gland that controls splenic tissue. The evidence is based on the fact that removal of the pituitary gland is followed by progressive atrophy of the splenic tissue. This atrophy is much greater than the loss of body weight and is comparable to the atrophy of the adrenal glands or the thyroid gland after hypophysectomy. In the hypophysectomized animal the capacity of splenic tissue to regenerate, as determined after removal of most of the spleen, is completely lost, so that the stump of the spleen is smaller at the end of a month than it was at first, whereas in the normal animal the spleen regenerates after partial ablation and gradually attains one-third or one-half the size of the normal spleen. Extracts of the anterior lobe of the pituitary gland have a marked stimulating effect on the spleen in normal animals, and my associates and I have been able to show that this stimulating factor is in the alkaline fraction of the extract of the anterior lobe, that it is separate from the thyrotropic substance and from the adrenotropic substance and that an extract rich in thyrotropic substance contains no splenotropic substance. After a period of ten days following the injection of the alkaline extract of the anterior lobe of the pituitary the spleen may increase to two or three times the normal size. Not only the spleen enlarges, but also the hemoblastic tissue of the bone marrow and the reticular tissue of the lymph nodes.

Concerning the indications for splenectomy, I should say that it is safe to perform splenectomy in cases in which the spleen either has lost its function through tremendous injury or in which experience has shown that there would be an unquestionable beneficial effect from its removal, such as in cases of hemolytic icterus or hemorrhagic purpura and sometimes in cases of Banti's disease. The beneficial effect in other conditions is transient and is due to the compensatory reactions stimulated by the removal. In hemorrhagic purpura there is a tremendous increase in the outpouring of platelets from the bone marrow. In Gaucher's disease removal of the spleen may modify the hemorrhagic crises. Otherwise, I think that removal of the spleen is contraindicated. Certainly in the tropics, in cases of protozoan infections, in which removal has been tried for cosmetic reasons because the spleen becomes so large, the issue is invariably fatal.

DR. MAURICE RICHTER: At the Seventy-First Annual Meeting of the British Medical Association in 1903, the Section of Pathology held a symposium on the subject of the pathology of splenic anemia. After listening to several papers

the president of the section summarized the subject by stating that it was obvious that the pathology of splenic anemia was wrapped in obscurity. In the last thirty odd years considerably more is known about the pathology of splenic anemia than at that time, because it is now known that splenic anemia does not exist. The term is gradually disappearing from the pathologic literature, and its place, to some extent, has been taken by the term Banti's disease. A great deal has been written on the relation of Banti's disease and splenic anemia, and I am glad to hear that all the speakers agree that Banti's disease does not exist either. If there were only a few other varieties of splenomegaly that did not exist the question of classification would be simple, but I think that for some time a considerable number of types of splenomegaly will have to be dealt with, the classification of which will be difficult. The various criteria for classification that Dr. Klemperer gave are clear, and they are useful for two reasons. In the first place, it is possible for the pathologist to follow the classification, and, in the second place, the various criteria which he used for placing disease in one or another category are based more or less on objective findings, and they draw on the imagination to a minimal extent. I noticed, however, that in the classification of the splenomegalies that Dr. McIntosh has given us there are several types which Dr. Klemperer did not mention. Most of these, of course, were omitted only because of lack of time. There are one or two, however, which I rather wish that Dr. Klemperer had mentioned in more detail. One is the splenomegaly due to the pathologic changes of the spleen in sickle cell anemia. This is of interest because it is one of the few conditions in which the pathologist can make a diagnosis that is satisfactory to himself and to the clinician as well. One is accustomed to recognize the pools of blood around the splenic follicles and the sickle shape of the cells in preparations of solution of formaldehyde as rather characteristic, whether or not one regards them as the result of malformation of the splenic sinuses.

The other condition which was present in Dr. McIntosh's classification and which was absent in Dr. Klemperer's is the last on the list: splenomegaly of undetermined origin. That, of course, is the bugbear of the pathologist and the clinician as well, and it would be interesting to hear a pathologist try to classify splenomegaly of undetermined origin on the basis of the etiology. In the absence of a satisfactory etiologic classification and in the absence of the exact knowledge of many of the functions of the spleen, the best that one can do is to consider purely objective findings: There is, or there is not, congestion; there is, or there is not, hyperplasia of one or the other element of the spleen; these are useful criteria.

Regarding other, rather minor specific points, I should like to say that in the histologic study of the spleen, either at autopsy or at operation, I have found it useful to use the smear method in connection with the usual histologic preparations. If one prepares an emulsion of spleen or makes imprints from the cut surface of the spleen it is possible to study the morphologic features of the cells with the methods that one is accustomed to use for ordinary blood smears. With these methods certain morphologic details in the cells are brought out more clearly than by the section method, thus enabling one at times to identify groups of cells and individual cells that are not readily recognized in the ordinary section.

DR. NATHAN ROSENTHAL: I feel that a clear and concise review of the subject has been given and that the previous speakers have left little for further discussion. All that I can do is to take up a few points which they probably did not have time to cover or to repeat some of those already taken up.

About twenty-two years ago I saw the first splenectomy for splenomegaly associated with hematemesis, at the Mount Sinai Hospital. This led me to study cases of Banti's disease, both in children and in adults, and I have come to the conclusion that this condition cannot be a clinical entity. There are patients with splenomegaly who have gastric hemorrhages, the type of condition described by Osler, and there are other patients with splenomegaly, leukopenia and a low platelet count who do not have gastric hemorrhages. These cases correspond somewhat with those described by Banti, who never described cases in which

there were gastric hemorrhages. In the last group and also in certain other cases the clinical picture may be dominated by cirrhosis of the liver.

The blood picture does not differentiate all these groups. It shows uniformity: anemia, leukopenia and thrombopenia. In still another unusual group the symptoms may be similar, but the blood picture may not show any changes with respect to the platelets. The postoperative course following splenectomy in the latter group is rather stormy, with generalized thromboses. The operation may be followed by persistent or intermittent thrombopenia. The pathologic examination of the spleen has not helped in differentiating these various types of splenomegaly.

In a study of splenomegaly it is important to keep in mind the following:

1. The various clinical pictures.

2. The changes in the blood.

3. Possible further differentiation by means of sternal puncture. The latter is becoming increasingly important. It is most useful in Gaucher's disease, Niemann-Pick's disease, polycythemia, hemolytic icterus, thrombocytopenic splenomegaly and malaria. Biopsy of material removed by Sternal puncture should be done in cases in which thrombopenia is suspected in order to determine the relative number of megakaryocytes. These are markedly increased in number in thrombocytopenic splenomegaly, the type of splenomegaly associated with postoperative thromboses.

4. Before splenectomy is performed the liver function should be studied by means of bromsulfalein, the Ara-Takata test and a galactose test. Evidence of damage to the liver contraindicates splenectomy. In fact, splenectomy should not be done before these tests are carefully carried out.

DR. PAUL KLEMPERER: There is one point which is worth reemphasis, now that it is agreed that the diagnosis of splenomegaly is possible from a purely morphologic standpoint: It is not easy to make a pathogenetic classification. Nevertheless, this must be the aim of the physician. He will accomplish this only with the full cooperation of the clinician and the pathologist, with the aid of the physiologist, who will point the way to new tests and new methods for the clinical approach. In this respect I feel that the reference by Dr. Perla to his work indicating a relation between the pituitary body and the spleen is of great significance. It seems to be significant not only in regard to the problem of splenomegaly but also in regard to diseases of the mesenchyma in general, among which splenomegaly is only a part, because I think that one can possibly best regard the spleen as concentrated mesenchyma. Therefore, one can probably study the functions of the mesenchyma in general best in the spleen.

In regard to the point of classification, this was as troublesome for me as it was for Dr. McIntosh. I considered all sorts of classifications given in the literature, and not one was satisfactory, either from the purely diagnostic standpoint or from the logical point of view. The difficulty is that these classifications are based on pathologic and morphologic criteria, on the one hand, and on clinical criteria, on the other hand. The pathologist, however, has to employ chiefly one principle, and I think that for the present one must continue to employ the morphologic criteria. It is interesting that every one of the speakers agrees that Banti's disease and splenic anemia do not denote a definite condition. It is then noteworthy that the term splenic anemia is still used in the "Quarterly Cumulative Index Medicus" and even includes Gaucher's disease.

Dr. Richter brought up the question of sickle cell anemia. I did not know whether I should include it or not, because the spleen is not always enlarged in sickle cell anemia; sometimes it is small. But if one wishes to include it, it belongs in the same group as hemolytic jaundice, with which it shows great similarity in the histologic picture.

Book Reviews

An Analysis of the De Generatione Animalium of William Harvey. By Arthur William Meyer, Professor of Anatomy, Stanford University. Price, $3. Pp. 167, illustrated. Stanford University, Calif.: Stanford University Press, 1936.

Every angle of the work of Harvey has been given exhaustive consideration except his "Exercitationes de generatione animalium," published in 1861. The many Harveian orations give it only scant notice. The fruit of observation and experimentation of many years "the book has undoubtedly deserved a better fate than to have been so long neglected." Meyer's analysis goes far to make up for this neglect. He first describes the editions of "De generatione." The illustrations show the title-pages and frontispieces of various editions. A good idea of the contents and plan of the analysis is obtained from the headings of the chapters: "The Times and Their Temper in Harvey's Days," "Some Embryological Predecessors and Contemporaries," "The Nature of De Generatione," "Philosophical Preconceptions," "Harvey's Attitude Toward Spontaneous Generation," "Harvey's Ideas Regarding Fertilization," "Ex Ovo Omnia," "The Generation of the Chick," "Development in Mammals," "The Lacteals and Some Other Matters," "Opinions of De Generatione." There are a valuable list of references and a good index. The impressive dictum "omne vivum ex ovo" frequently attributed to Harvey is a "famous misquotation" with a complicated history, which is well analyzed by Meyer. The nearest Harvey came to this dictum appears to be the heading of his "Exercise the Sixty-Second": "ovum esse primordium commune, omnibus animalibus." Meyer's purpose "to analyze the treatise and to stimulate a re-examination of it" has been accomplished most admirably. The book is a fine contribution to the full understanding of Harvey. The product of true scholarship, it appears in a pleasant and appropriate form. A great difficulty in assaying the true worth of Harvey's "De generatione" is the tendency to do so in the light and under the influence of subsequent developments. Meyer has escaped this danger by heeding Harvey's own admonition: "Give me leave therefore to whisper this to thee (friendly Reader) that thou be sure to weigh all that I deliver in these Exercitations, touching the Generation of Living Creatures, in the steady scale of experiment; and give no longer credit to it, than thou perceivest it to be securely bottomed, by the faithful testimony of thy own eyes."

Experimental Epidemiology. By M. Greenwood, A. Bradford Hill, W. W. C. Topley and J. Wilson. Medical Research Council Special Report Series, no. 209. Price, 3 shillings, sixpence. Paper. Pp. 204. London: His Majesty's Stationery Office, 1936.

This notable summary of experimental and statistical work extending over some fifteen years and involving the use of between 100,000 and 200,000 mice is clearly and admirably written, and will be read eagerly by all interested in the general phenomena of infectious disease. It represents one of the most important attempts ever made to increase knowledge of the essential nature and causation of epidemics. Here one will find well documented, and with all the available data subjected to a searching statistical analysis, the results of a series of studies on the general character of the epidemic process in herds of mice. There is a well balanced discussion of the effect of such factors as the continuous and the intermittent migration of "susceptibles" into herds of mice in which infectious disease is prevalent. The results of experimentation seem to show that the relative immunologic constitution of a population exposed to risk, as influenced by either the birth or the immigration of "susceptibles," determines to a large extent the periodicity of certain epidemic diseases. Numerous points of great practical importance are considered, such as the effect of artificial immunization on herd immunity, the probable influence of school closure on the spread of disease, the influence of bacteriophage on such a disease as mouse typhoid, and similar topics. The authors themselves were unable to obtain evidence that any change in steady diet exerted an influence on the group mortality. They also concluded that so far as present methods of

immunization are concerned there is no indication that a lowering of mortality is associated with an equivalent lowering of infection. They are conservative about applying to human epidemiology the generalizations derived from animal experimentation. They fully realize, as not all workers in this field have done, that the conditions obtaining in segregated herds of mice under experimental conditions must be cautiously interpreted in their relation to problems of human epidemiology. Altogether this publication is a well balanced, stimulating contribution to what older writers would have called the philosophy of the rise and fall of epidemic disease.

The Specificity of Serological Reactions. By Karl Landsteiner, M.D., The Rockefeller Institute for Medical Research, New York. Cloth. Price, $4. Pp. 178. Springfield, Ill.: Charles C. Thomas, 1936.

Although the phenomenon of serologic specificity has been the subject of numerous investigations during the past third of a century, it still remains one of the most baffling as well as most fascinating problems in the whole field of serology. In this monograph, which is a revision of a work originally published in German in 1933, the author has collected the important work bearing on this fundamental problem. The book contains six chapters, dealing with introductory matters, the serologic specificity of proteins, the specificity of cell antigens, the specificity of antibodies, artificial conjugated antigens and chemical investigations on specific cell substances. At the end of each chapter, there is a comprehensive bibliography listing hundreds of papers covering the subject matter of that chapter. The chapter on artificial conjugated antigens and serologic reactions with simple chemical compounds is especially valuable because of the very concise discussion of the author's fundamental work in this field. Because of the author's prominence as the outstanding student of the subject and the many hundreds of references to the literature which this book contains, it should find a welcome place in the libraries of all workers interested in the problems of serologic specificity.

Bernhard Bang Selected Works. Edited by Vald. Adsersen, Professor at the Royal Veterinary and Agricultural College in Copenhagen. Paper. Price, 30 Danish crowns. Pp. 560, with 6 plates. Copenhagen: Levin & Munksgaard, Einar Munksgaard. London: Humphrey Milford, Oxford University Press, 1936.

Bernhard Bang, the great Danish veterinary pathologist and bacteriologist, was born in 1848 and died in 1932. Shortly before his death the Danish publisher of this volume proposed to him the publication "in the chief languages" of a selection of his most important scientific papers. Nothing came of this proposal, however, and the present selection is the work of the editor. There are twenty-six articles in the book, thirteen in German, ten in English and three in French. Six of the articles have been translated from Danish, one into German and the others into English. The articles fall into three groups: The first group represents Bang's work on actinomycosis, mastitis, local infectious necrosis, endocarditis in swine erysipelas, epizootic or infectious abortion, Johne's disease and abortion due to tuberculosis. There are two articles on infectious abortion, both published originally in English, and the earlier one (1897) contains the description of the causative organism (Bang's bacillus, Brucella abortus-bovis). The second group of articles consists of three on tuberculosis of the udder and on tuberculous milk. These three articles embody the chief results of Bang's work on those subjects. The third group contains a selection of Bang's writings on tuberculin and its use as advocated by him in the control of tuberculosis in cattle. There is a biographic sketch of Bang by the editor which gives a good review of Bang's career and a good idea of his personality. The frontispiece shows Bang as he appeared in 1920. The publisher's work is well done; the paper is first class, and the print is easy to read. The issue of the book was made possible by a grant from the Rask-Örsted Foundation. Bang is an outstanding figure in veterinary science. He made original contributions of fundamental significance to our knowledge of important diseases. "Bernhard Bang Selected Works" will be a welcome and useful addition to the libraries in his field.

Books Received

EPIDEMIC AMEBIC DYSENTERY. THE CHICAGO OUTBREAK OF 1933. U. S. Treasury Department, Public Health Service. National Institute of Health Bulletin, no. 166. Paper. Price, 20 cents. Pp. 187, with 35 illustrations. Washington, D. C.: Government Printing Office, 1936.

THE INFLUENCE OF DIET ON CARIES IN CHILDREN'S TEETH (FINAL REPORT). Committee for the Investigation of Dental Disease (Assisted by Alan Deverall and Mabel Reynolds). Medical Research Council, Special Report Series, no. 211. Paper. Price, 2 shillings. Pp. 137. London: His Majesty's Stationery Office, 1936.

ANNUAL REPORT OF THE INSTITUTE FOR MEDICAL RESEARCH FOR THE YEAR 1935. A. Neave Kingsbury, Director, Institute for Medical Research, F. M. S., Kuala Lumpur, Federated Malay States. Paper. Price, 50 cents. Pp. 126. Kuala Lumpur: Federated Malay States Government Press, 1936.

INVESTIGATIONS ON RESPIRATORY DUST DISEASE IN OPERATIVES IN THE COTTON INDUSTRY. C. Prausnitz. Medical Research Council, Special Report Series, no. 212. Paper. Price, 2 shillings, sixpence. Pp. 73, with 26 illustrations. London: His Majesty's Stationery Office, 1936.

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CORRECTION

In the article by Drs. Clark, Graef and Chasis entitled "Thrombosis of the Aorta and Coronary Arteries with Special Reference to the 'Fibrinoid' Lesions," in the August issue (**22**:183, 1936), the word "fibrinous" in the twenty-second line on page 185 should read "fibrous."